ENVIRONMENTAL TIMING AND CONTROL OF REPRODUCTION IN THE POWAN OF LOCH LOMOND, COREGONUS LAVARETUS (L.) (TELEOSTEI) IN RELATION TO ITS PINEAL ORGAN

William David O'Connell

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Environmental timing and control of reproduction in the powan of Loch Lomond, *Coregonus lavaretus* (L.) (Teleostei) in relation to its pineal organ.

By

William David O'Connell

Department of Zoology, University of St Andrews

Thesis submitted for the Degree of Doctor of Philosophy

November, 1984

DECLARATION

I William David O'Connell hereby certify that this thesis which is approximately 35,000 words in length has been written by me, that it is the record of work carried out by me, and that it has not been submitted in any previous application for a higher degree.

date...23.-.11-..84.. signature of candidate...[redacted].....

DECLARATION

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate to the degree of Ph.D. of the University of St Andrews and that he is qualified to submit this thesis in application for that degree.

date 23.11.84 signature of supervisor [redacted]

DECLARATION

I was admitted as a research student under Ordinance No.12 on 1—10—78 and as a candidate for the degree of Ph.D. on 1—10—79.; the higher study for which this is a record was carried out in the University of St Andrews between 1978 and 1981.

date 23—11—84 signature of candidate [redacted]

Abstract

The reproductive cycle of Coregonus lavaretus (L.) in Loch Lomond was investigated by monthly sampling. The stages of the cycle occurred at the same time each year and were precisely timed. The environment followed a regular pattern which varied seasonally and was repeated annually. Spawning synchrony within the population during the short breeding period may be in response to lunar phases.

A qualitative echosounding survey was made. The spatial distribution of the fish is probably related to their feeding behaviour and was mainly pelagic in summer and benthic in winter. Diel vertical migrations were recorded at dawn and dusk and appeared to be related to negative solar altitudes. The fish occurred at the surface during the night and persisted with their diel vertical migrations when feeding behaviour was benthic.

The regulation of the reproductive cycle in the common sole Solea solea was investigated. The timing of spawning is ultimately determined by sea temperature, and spawning synchrony within the population is probably achieved in the initiation of exogenous vitellogenesis by a unified response within the breeding population to a stimulatory photoperiod.

In both Solea solea and Coregonus lavaretus, initiation of exogenous vitellogenesis occurred during a rapid rise in the condition of the fish. The photosensitivity of the reproductive system may possibly be linked to a threshold condition.

The pineal organ of Coregonus lavaretus is typically salmonid and the convoluted epithelium contained photoreceptors, interstitial cells, and neurones. The interstitial cells gave rise to processes which extended into the perivascular space. Photoreceptor cells synapsed with neurones, photoreceptor cells (lateral processes) and possibly other cell types. The results suggest that the pineal organ functions as a photoreceptor.

CONTENTS

General Introduction

Seasonal reproduction is the normal behaviour in mid - high latitudes for most teleosts, and there is evidence to suggest that seasonality exists in some deep sea fish [< 2000 metres] (Gordon, 1979) and tropical species (Scott, 1974; Munro, 1973; Lowe-McConnell, 1979). Baker (1938) provided the theoretical basis for the evolution of seasonal reproduction. He suggested that the ultimate causes for a species having a defined breeding season are optimal conditions for the development of offspring. The stimuli that cause the parents to reproduce at the appropriate time are the proximate factors.

The female reproductive cycle of many teleost species will recrudesce in captivity, but often fails at the onset of final maturation, ovulation or spawning (Scott, 1979). The reasons for failure can be divided into three areas; the inhibition of social behaviour by overcrowding and lack of space for the expression of courtship behaviour; the accumulative effects of poor husbandry; inadequate simulation of the natural environment of the species. The first and second causes are relatively easy to rectify, but the third requires an understanding of the interaction between the reproductive cycle and the environment. In particular, it is necessary to identify the nature of the proximate cue. Although there are many studies on the regulation of reproduction in teleosts (reviewed Schwassmann, 1971; de Vlaming, 1974; bibliography Htun-Han, 1977), very few relate results to the natural environment of the experimental animal.

The artificial induction of final maturation and ovulation is now commonplace in the culture of teleosts (Harvey & Hoar, 1979), but there are many associated problems. Repeated treatments with exogenous proteins may trigger an immune response; regular handling can be stressful and interfere with hormonal action; the identification of sexes outwith the breeding season may be difficult (Lam, 1982). The cost of hormones, availability,and restrictions pertaining to their use, may make induction

techniques impracticable for many workers. An understanding of the proximate cues required by a species for the successful completion of the reproductive cycle could replace the need for artificial induction techniques, or complement their use; such an understanding would reveal periods when fish may be refractory to hormonal stimulation.

Research on the reproductive cycles of mid to high latitude teleosts is partially inhibited by the limited availability of experimental animals; the individual stages of the reproductive cycle occur only once every year. Environmental manipulation of the reproductive cycle can create all year round spawning groups (Bye & Htun Han, 1979), which benefits research and has commercial application. However, the precision of such control depends, to a large extent, on understanding the timing requirements of the species. These requirements are ultimately dictated by the reproductive strategy employed. The timing of reproduction involves various physiological systems and therefore its understanding requires an integrated approach (Scott, 1979).

Evidence from experiments on teleosts suggest that the pineal organ influences many physiological systems including reproduction; there is some indication that pinealectomy can be stimulatory or inhibitory to gonad development at different times of year (de Vlaming, 1982). However, the evidence that the teleost pineal is involved in the regulation of reproductive cycles is not wholly convincing. Many workers have failed to take into consideration in their experimental design (a) the effect of seasonal variation in responsiveness to environmental factors or (b) made allowance for the effect of an endogenous circannual reproductive rhythm. The role of the pineal organ remains to be elucidated.

This investigation forms part of a current research programme into the control of reproduction in the powan of Loch Lomond, Coregonus lavaretus (L.). The aim of this study is to assess the environment and behaviour of the powan, in relation to its reproductive biology, and to

gain an understanding of the factors involved in the fine timing of the reproductive cycle. The work is divided into four chapters:

Chapter 1 A study of the environment of the species, and how it varies throughout the year, coupled to regular sampling of the population to assess its reproductive state.

Chapter 2 A qualitative echosounding study, to investigate the spatial and temporal distribution of the powan.

Chapter 3 An experimental investigation into the regulation of the reproductive cycle.

Chapter 4 A morphological and ultrastructural study of the pineal organ.

CHAPTER 1

The Reproductive cycle

Introduction

The whitefish Coregonus lavaretus (L.) and the vendace Coregonus albula (L). are species of economic importance in northern Europe and extensive fisheries have developed. Both species become readily adapted to changes in the environmental conditions. They compete to only a small degree with other valuable species and this has enabled their introduction into many lakes and reservoirs. A large amount of data exists on the spawning of both species and this has been reviewed by Zuromska (1982). The spawning sites and behaviour of the species are very similar. They can spawn at different latitudes at similar times or at completely different times at the same latitude. The range of spawning period extends from October to March but is mainly divided into autumn (October - November) and winter (December - January) groups. The duration of the spawning period does not exceed 2-3 weeks and the date of the beginning of spawning can vary by as much as 2 weeks in different years. However, data on the reproductive cycle and the cues used by the fish to time their spawning activity is lacking.

The reproductive cycle of salmonids can be influenced by photoperiod manipulation (reviewed, Scott & Sumpter, 1983). Recent research indicates that it is the long daylengths experienced by rainbow trout Salmo gairdneri during the spring and early summer which may initiate gonad recrudesence. Most studies however are based on salmonids maintained in captivity and there is a lack of basic data on the environment and reproductive cycle of natural populations.

The need for data from natural populations is stressed by Scott (1979). In a series of environmental control experiments on the minnow Phoxinus phoxinus he demonstrated that the proximate cue which initiated exogenous vitellogenesis was a photostimulatory photoperiod; however, the exposure

to the photoperiod was the result of a behavioural change induced by a water temperature of 8 to 9°C. It was therefore temperature which ultimately dictated the synchrony of this physiological phase within the breeding population and the time of final maturation. Moreover, he found that in the absence of a natural photoperiod the fish continued to reach sexual maturity although this was delayed. The importance of photo-stimulation in the timing of teleost reproductive cycles may refer more to the experimental rather than the natural situation and caution is therefore required in interpreting the results of laboratory based experiments (Scott, 1979).

The processes involved in the building of gonad, especially ovary, are complex and must involve the co-ordinated activity of physiological systems other than reproduction. The energy demands of recrudesence are high and there may be periods when the gonad grows so rapidly that dietary intake cannot cope and matter must be diverted from other tissue (Ursin, 1979). It seems reasonable therefore to expect fish, especially females, to attain a certain condition before beginning the most energy demanding stages of the reproductive cycle. Reshetnikov et al (1970) found that the onset of sexual maturity in whitefish was associated with the attainment of a definite level of fat reserves. It may be therefore that dietary intake is involved in the timing of reproductive cycles. Many captive populations are fed on a regular basis, which does not simulate the cycle of food availability in the wild. The condition of captive fish is likely to be different from wild fish and may possibly represent another source of error in laboratory based environmental control experiments.

Environmental factors can interact with the hypothalamo-pituitary-gonad axis to influence the timing of reproductive development; serum gonadothrophin (GtH) levels increase with rising temperature and therefore thermal acceleration of vitellogenesis may be related to increased circulating GtH (Crim, 1982). A diel rhythm, phased with the light-dark

cycle, in the level of pituitary GtH in trout has been described (O'Conner, 1972).

There appears to be a need for workers to define the natural cycle of their experimental animal, prior to embarking on detailed studies of the finer points of teleost reproductive physiology. This investigation therefore seeks to assess the environment and behaviour of the powan Coregonus lavaretus in relation to its reproductive biology and as a preliminary to a more detailed investigation of the important phases in the regulation of the reproductive cycle.

Materials and Methods

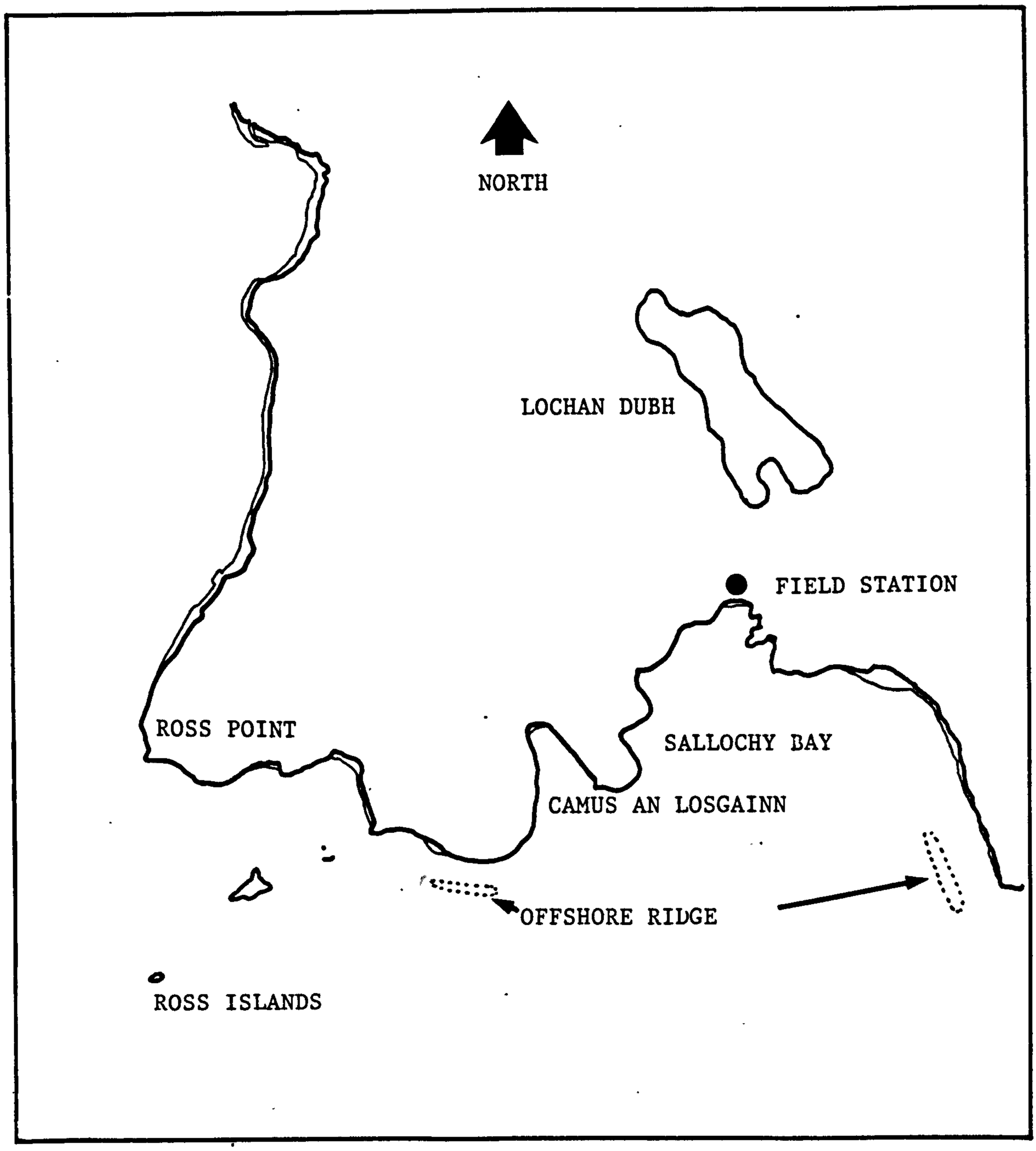
The species chosen for this study was Coregonus lavaretus (Linnaeus) = Coregonus clupeoides (Lacépède), commonly known as the powan of Loch Lomond, Scotland. A subfamily of the Salmonidae, the coregonines are mostly anadromous and boreal in distribution. Britain retains a number of glacial relict populations which are now landlocked in freshwater lakes surrounding the Irish Sea : Loch Eck (powan), Castle Loch and Mill Loch (vendace), Lake district (schelly), Bala lake = Llyn Tegid (gwyniad), Lough Neagh and Lough Erne (pollan). Coregonines are of particular economic importance throughout their range but have not been exploited commercially in Britain for over forty years. The Loch Lomond population is therefore especially suitable for research purposes as it remains essentially unexploited and can be caught in large numbers throughout the year.

Sampling was carried out at the north end of Sallochy Bay, mainly in the region of Camus an Losgainn, and on the spawning ground between the Ross Islands (Fig. 1). The gill nets used were standard commercial nets of No. 0 nylon thread, 39 mm (knot to knot) supplied by Norsenet, Bergen, Norway. The nets were used in gangs, containing up to 8 sections (1 section = 25m.). Nets, unless set between the Ross Islands (rock and gravel) or in small bays (sand and gravel),were on steep slopes (thick silt) to 30m. The nets set between the Ross Islands spanned the entire gap (200m). The gill nets were generally set between 15.00 - 21.00 hours and lifted between 09.00 - 14.00 hours the following day. Sampling was carried out at regular intervals throughout the year (June excepted) and was intensified in the period before spawning was due to begin.

Standard measurements of each fish were taken: total length, wet weight gonad weight and pineal weight, and are defined below:

Total length: length to the nearest millimetre from the tip of the lower jaw to the tip of the caudal fin in a position of maximum

FIGURE 1 The sampling area


NORTH
LOCHAN DUBH
FIELD STATION
ROSS POINT
SALLOCHY BAY
CAMUS AN LOSGAINN
OFFSHORE RIDGE
ROSS ISLANDS

extension.

Wet weight: weight to nearest gram of intact fish with excess liquid removed from surface.

Gonad weight: weight to nearest 10 mg of gonad dissected from the body.

Somatic weight: total weight minus gonad weight.

Pineal weight: weight to nearest µg. of pineal dissected from body. To minimise the effect of dessication, the tissue was weighed within 30 seconds of removal from the body (Mettler ME 30 micro-analytical balance).

The gonadosomatic index is the gonad weight expressed as a percentage of total body weight (wet).

The condition factor and somatic condition factor:

$$CF = \text{weight}/\text{length}^b$$

The value of b has been shown to be close to 3 in all salmonid species and satisfactory for coregonines (Deason and Hile, 1944). Log/log plots of total weight against length for powan showed that there is little change in the relationship throughout the year for males, but that there is a difference in the value of the exponent b between May and September in the female population. This is probably due to the rise in condition which occurs at this time. For the purpose of this study, the power relationship of 3 was taken to represent the average value for the year.

$$SCF = (\text{Total weight} - \text{gonad weight}) / \text{Length}^3$$

Visual inspection of the ovary during the spawning period enabled the reproductive state of females to be assessed:

Preovulating: ova yellow-orange, opaque, and still in ovarian membrane.

Ovulating: ova translucent orange, and still in ovarian membrane.

Ovulated: translucent orange ova free in coelom.

Spent: ova practically all lost from coelom.

Light transmission An attempt was made to gain a general indication of light transmission in the surface layers over one year. It was intended to take readings at mid-day with a clear sky and undisturbed water but this proved impracticable. Wind and unsettled water interfered with measurements and cloud cover reduced light levels. Problems with the equipment prevented sampling on several occasions.

Irradiance measurements are made over a narrow spectral band. The advantage is that the measured value can be selected to approximate the spectral sensitivity of the animal. A green monochromatic (504 nm) filter was used for all measurements as the absorption maxima of coregonid visual pigment is quoted as 536 nm (Haram, 1968). Downwelling irradiance was measured with an S-20 KNaSb[Cs] negative electron photocathode which was sensitive in the range 300 - 900 nm (Responsivity - 300 μA/lm.), and contained within a lime glass dome. The instrument was protected from direct sunlight, and left in the loch water for several minutes to reduce temperature differentials before measurements were made. Readings were taken as the photometer was lowered to depth and as it was brought back to the surface.

The measurements made only give the most general indication of transmission and high accuracy was not sought. The immersion effect correction was not made, and the angular collecting response was not established (Smith, 1969). The technical aspects of photodetection and negative electron affinity photocathodes are discussed by Dennis (1979).

Water level The data on water level in Loch Lomond is given in metres above ordnance datum. The information was provided by the Clyde River Purification Board, Glasgow.

Results

Females

The mean gonadosomatic index, gonad weight and condition factors varied seasonally and followed a cyclical pattern. The various stages of the reproductive cycle occurred at the same time each year and were precisely timed.

The mean gonadosomatic index increased from mid-summer to a yearly maximum of about 20 during the period immediately preceding spawning (Fig.2 The highest value recorded for a female was 23.89; ovary weight was 152.4g and total body weight 638g. After spawning there was a period of about two months before the gonadosomatic index fell to a basal level of around 1.2 . The standard deviation in the mean value from March to May was lower than at any other time in the reproductive cycle (Fig. 2). From July until spawning the standard deviation in the mean value increased as individual variation in the rate of development (vitellogenesis) became apparent.

The mean gonad weight increased from July until spawning but there was some indication that the rate levels off from 19 December 79 (Fig. 4). In Loch Lomond Coregonus lavaretus is known to spawn from early December (Scott, per com.). It may be possible that the fish were primed to spawn from early December each year but must wait for a cue to initiate final maturation and ovulation; during the waiting period the rate of exogenous vitellogenesis slowed down. Exogenous vitellogenesis has been shown to begin during July and endogenous vitellogenesis from April (Rashid, 1984).

The condition factor and somatic condition factor were closely related from February until mid to late July each year (Fig. 6). Both factors reached their lowest value during May when the mean value was around 0.67. Between mid-May and mid to late July the condition factors increased rapidly. Unfortunately it was not possible to sample the population during this period but as the rise was considerable it is assumed that it probably began during late May or early June. The fish are known to feed extensively

on plankton at this time (Slack et al, 1957). From August until spawning the condition factor was maintained, with a mean value between 0.8 and 0.9. During this period the mean somatic condition factor fell progressively, presumably as material was diverted to the ovary.

The ovaries were paired. There was asymmetry in size between left and right ovaries. The ovaries of ripe, preovulating females were compact and the ova opaque. The inception of ovulation was marked by a reduction in the compactness of the ovary, and the ova became translucent. Actively ovulating females had translucent ova free of the ovary and expressible from the cloaca. After spawning, the ovary contained only primary oocytes, follicular calyces, and a few unspawned ova which underwent atretic resorption.

The beginning and end of the spawning period is a subjective assessment. It is not possible to be certain that spawning begins simultaneously throughout Loch Lomond as the spawning sites are widely dispersed. The beginning of spawning was based on the presence of ovulated and spent females caught in gill nets. On 22 December 77, fish recovered from an area away from known spawning grounds gave no indication that spawning had begun. Fish recovered from the Ross Islands on the same night included several ovulated and spent females. A sample of the population on 14 December 78 (near the field station) gave no indication that spawning had begun, yet many spent females were found at the Ross Islands between 16 to 18 December. The combined evidence from netting, echosounding, and a diving survey (see chapter 2) suggests that the Ross Island ridge (Fig. is a major spawning site; the best approximation for the beginning of spawning will therefore come from monitoring this site.

Spent females were recovered from the Ross Islands on 22 December 77, and the full moon reached its maximum phase of 1.00 on 25 December 77. The full moon occured on 14 December 78 and spent females were recovered from the Ross Islands between 16 to 18 December 78. Altogether, 256 fish

TABLE 1

The relationship between the initiation of spawning and the period of full moon.

1966-1967	Full moon 27.12.66	Spawning time indicated by Maitland (1968) to have just begun at the end of December 1966.
1969-1970	Full moon 23.12.69	Spawning was in progress at 31 December 1969 (Scott, per comm.).
1971-1972	Full moon 31.12.71	Spawning began in early January 1972 (Scott, 1979).
1976-1977	Full moon 6.12.76	Spawning began in early December 1976 (Scott, 1979).
1977-1978	Full moon 25.12.77	Spawning had begun on 22 December 1977 at the Ross Islands (this study).
1978-1979	Full moon 14.12.78	Spawning had begun on 16 December 1978 at the Ross Islands (this study).
1979-1980	Full moon 3. 1.80	Spawning had not begun on the 29 December but was underway by 12 January 1980.
1980-1981	Full moon 21.12.80	Spawning had not begun on the 21 December but was almost complete by 18 January.

were caught: 193 males (many with running milt), 22 spent females, 29 ovulated, and 13 preovulatory. During December 1979 a considerable effort was made to maintain sampling up until the full moon on 3 January. Unfortunately bad weather prevented the Ross Island site being sampled, and nets recovered on 29 December contained no spent or ovulated fish. The next sample on 11 January produced spent females. Table 1 shows the years for which information exists on the spawning period, and there appears to be a relationship between the occurence of a full moon and the beginning of spawning.

The spawning period of Coregonus lavaretus in Loch Lomond lasts for about four weeks, but the most intense spawning activity appears to occur within the first three weeks. On several occasions mature females turned up in samples several months after the spawning period had ended:

28. 2.80 mature female, ovaries 61.8g, preovulatory

16. 4.80 mature female, ovaries 80.5g, ova translucent orange and still in ovarian membrane.

16. 4.80 mature female, ovaries 40.1g, preovulatory

16. 4.80 mature female, ovaries 109.8g, ova translucent orange and still in ovarian membrane.

Zuromska (1982) reported mature female vendace Coregonus albula in mid-May, from a winter spawning population and Scott (per com.) found females six months out of phase with the main population (Loch Lomond), although these were very rare.

Males

The mean gonadosomatic index, gonad weight and condition factors varied seasonally and followed a cyclical pattern. The various stages of the male reproductive cycle occured at the same time each year and were precisely timed.

The gonadosomatic index fell after spawning, as unshed spermatozoa were resorbed, to a minimum in early July (Fig. 3). From early to mid-July

it proceeded to rise to a yearly maximum of 2 to 2.25 in September before falling gradually to December. Individual values could be higher; a 42.5 cm male had a gonadosomatic index of 3.29 (November, 1979), and the largest testes recorded weighed 17.9g from a 41.1 cm specimen (January, 1979). The largest fish recorded throughout the study was a male weighing 741g but the testes in this fish were very small (0.4g).

The mean gonad weight reached its lowest value in early July (Fig. 5). The first significant increase in gonad weight occurred during July at which time secondary spermatogonial development was observed in the tubule walls (Fuller et al, 1976). During the period from October to January the tubules were full of spermatozoa. The reduction in testis weight from October (Figs. 3,5) may be due to the small mass of spermatozoa relative to earlier spermatocyte stages (Scott, 1979). The rise in testes weight immediately before spawning (Fig. 5) was probably due to gonad hydration.

The condition factor and somatic condition factor were closely related throughout the year with the greatest difference between September and January (Fig. 7). The highest mean condition factor was recorded on 25 July 80 when it had risen to 0.90. The lowest value occurred during May. Unfortunately it was not possible to sample the population during the period from mid-May to early June when both condition factors rise suddenly and rapidly. As with the females it was assumed that the first significant increase in condition factors began during late May or early June when the fish are known to feed extensively on plankton (Slack et al, 1957). Unlike the females where the condition factor maintained its value from July until spawning, the condition factor gradually dropped from September or October (Fig. 7).

During the spawning period catches of powan between the Ross Islands comprised 90% males. Individual females may form unisexual shoals over deep water and move on to the spawning ground as they ovulate; the males congregate on the spawning grounds during the spawning period and possibly

FIGURE 2 Mean gonadosomatic index for females during the period 1977 - 1980. One S.D. above and below mean.

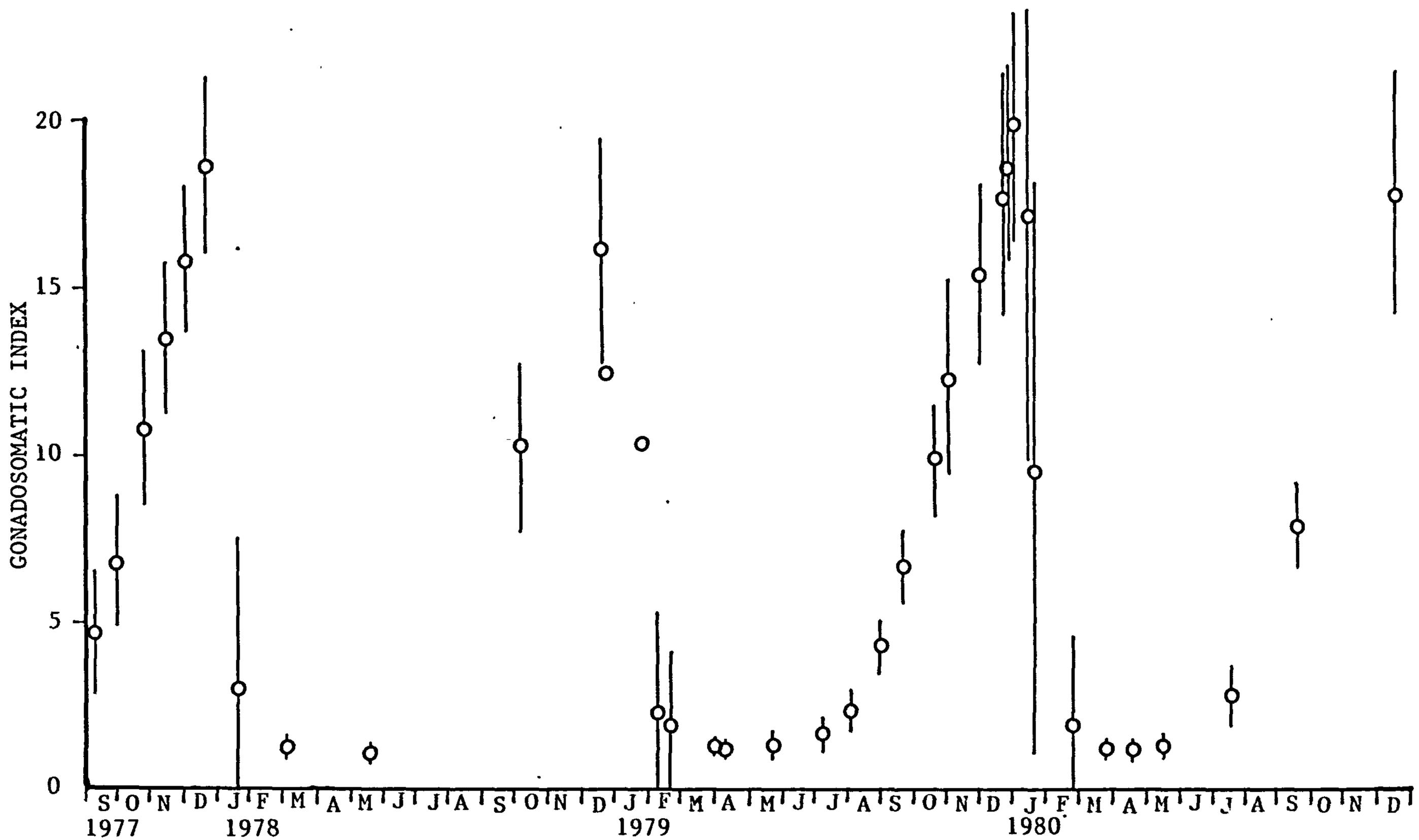

GONADOSOMATIC INDEX
20
15
10
5
0
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1977
1978
1979
1980
DATE

FIGURE 3 Mean gonadosomatic index for males during the period 1977 - 1980. One S.D. above and below mean.

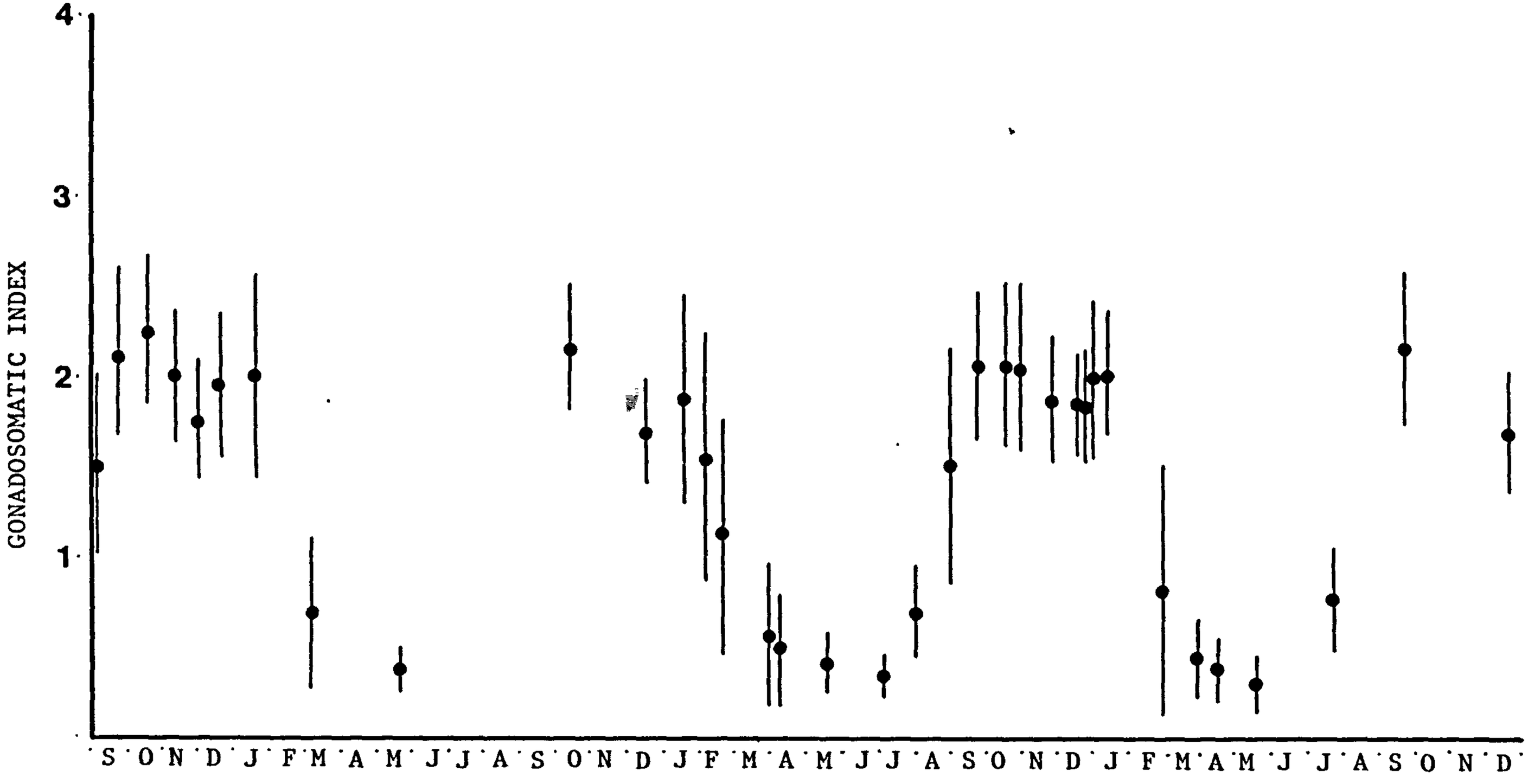
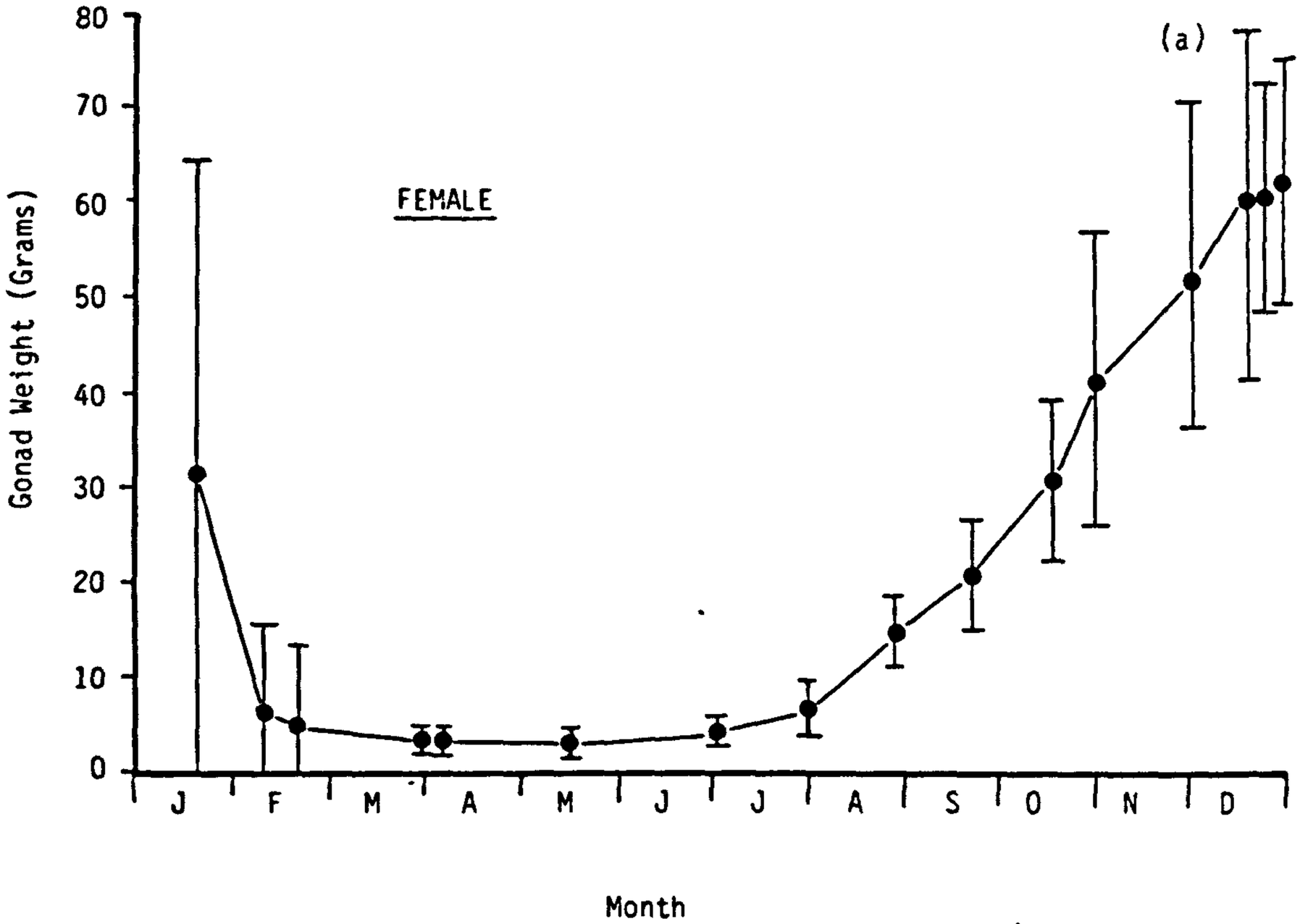

GONADOSOMATIC INDEX
4
3
2
1
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D

FIGURE 4 The mean gonad weight for female powan taken in gill nets near the spawning ground during 1979. One S.D. above and below mean.

FIGURE 5 The mean gonad weight for male powan taken in gill nets near the spawning ground during 1979. One S.D. above and below mean.


FEMALE
(a)
Gonad Weight (Grams)
80
70
60
50
40
30
20
10
0
J
F
M
A
M
J
J
A
S
O
N
D
Month

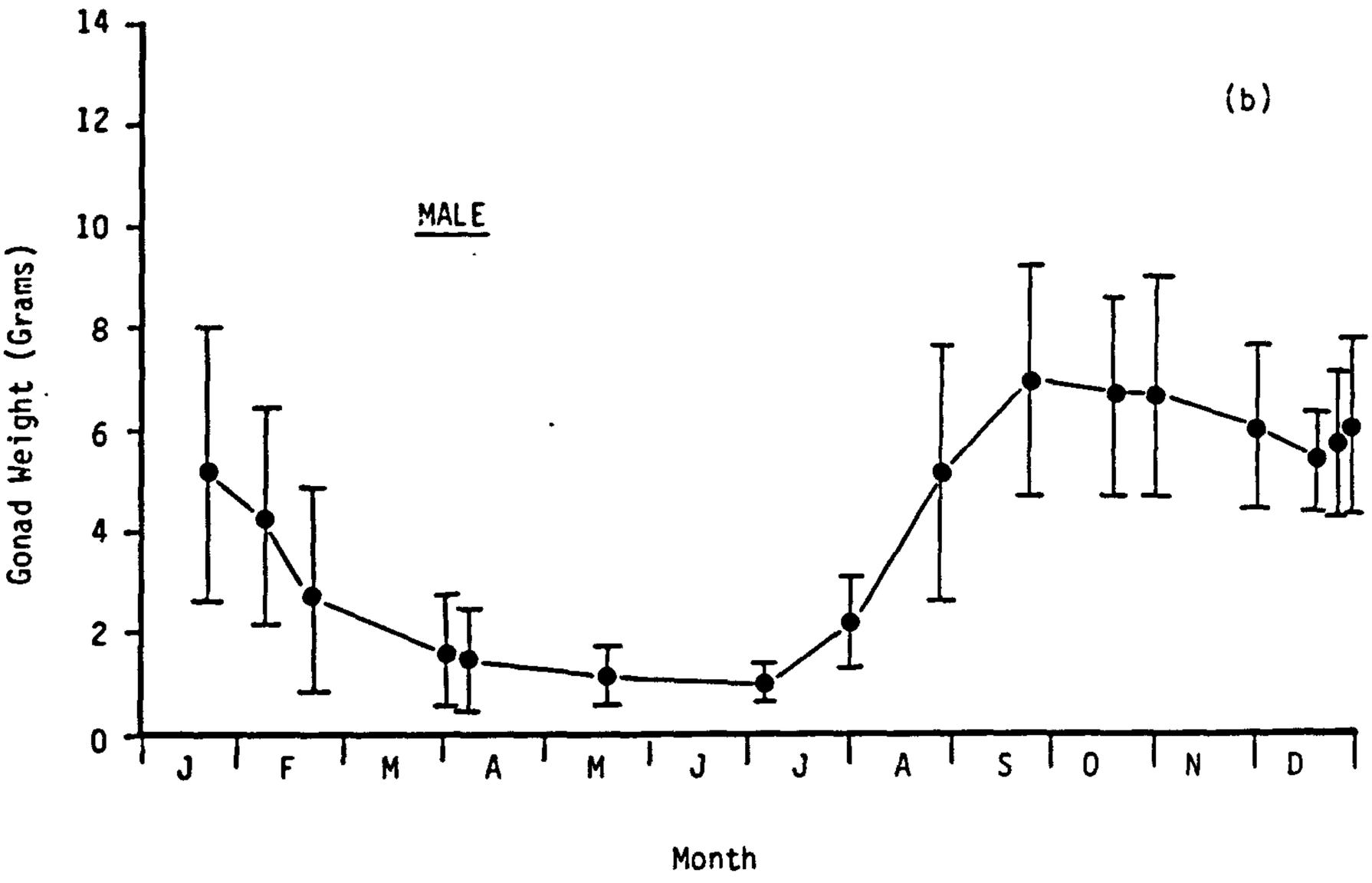

MALE
(b)
Gonad Weight (Grams)
14
12
10
8
6
4
2
0
J
F
M
A
M
J
J
A
S
O
N
D
Month

FIGURE 6 Mean condition factor (black dots), and somatic condition factor (open circles) for females during the period September 1977 - December 1980. One S.D. above and below mean.

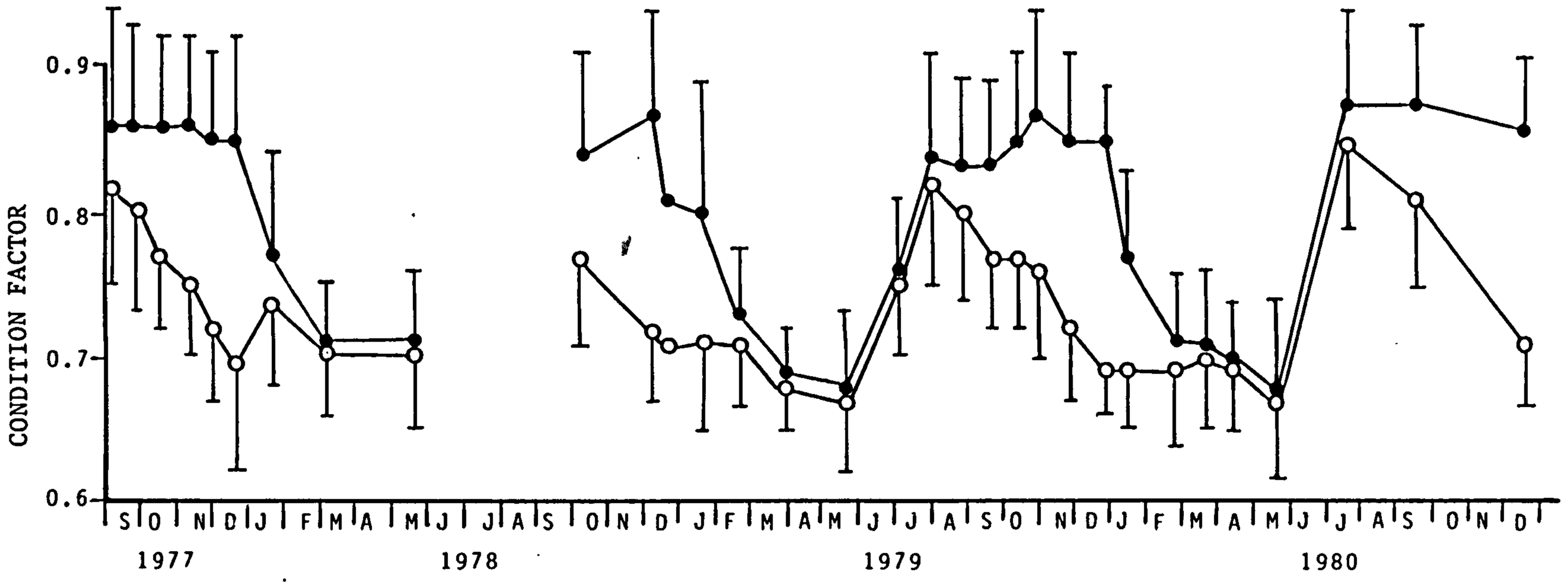
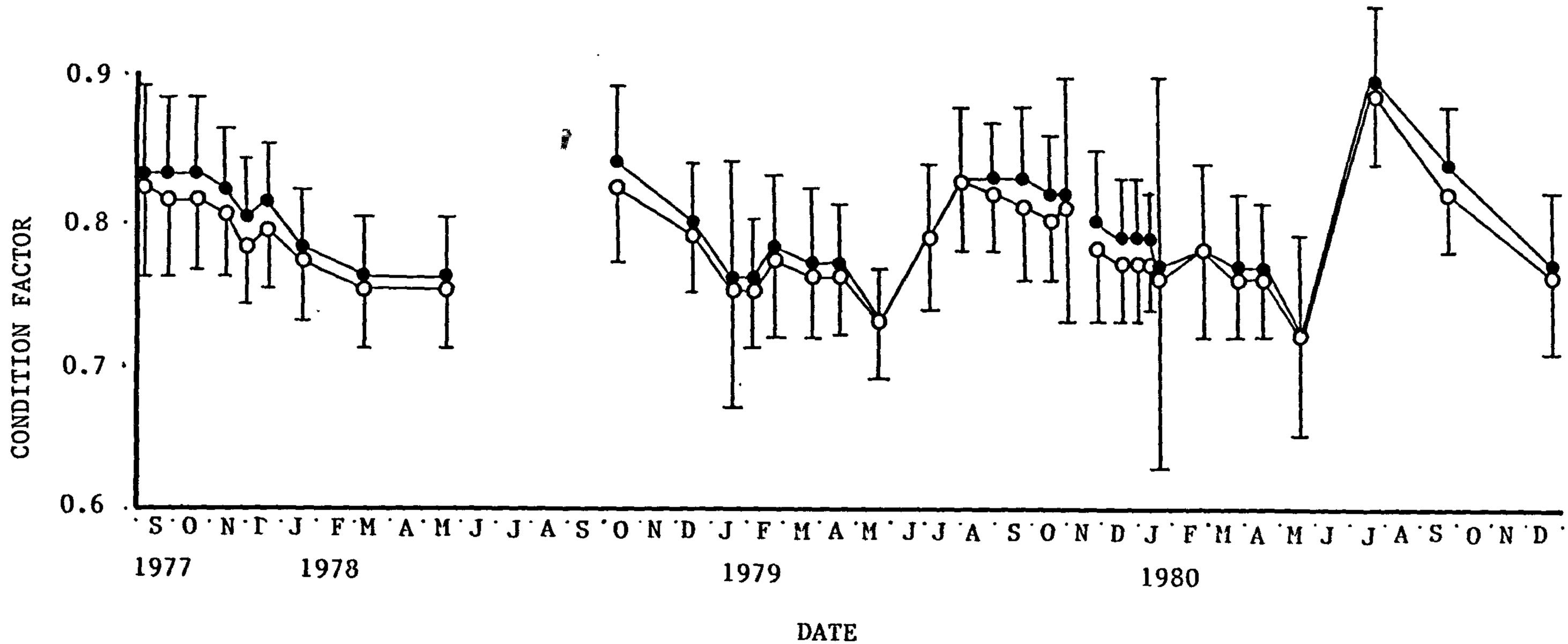

CONDITION FACTOR
0.9
0.8
0.7
0.6
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1977
1978
1979
1980
DATE

FIGURE 7 Mean condition factor (black dots), and somatic condition factor (open circles) for males during the period September 1977 - December 1980. One S.D. above and below mean.


0.9
0.8
0.7
0.6
CONDITION FACTOR
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1977
1978
1979
1980
DATE

some time before spawning (Fuller et al, 1976).

Pineal Organ

Pineal weights were recorded for one year and there is some suggestion of a yearly cycle (Fig. 8). The weighing equipment only became available towards the end of the study and there was no opportunity for a further year's sampling. The mean values ranged between 2.28mg in May to a maximum of 4.03mg during October. There was no indication of any significant variation in weight between the sexes.There is some suggestion of a relationship between body weight and pineal weight. Table 2 summarises the results.

Table 2

Date	x (mg)	SD	n	SE	Sex
5 March	2.49	0.70	17	0.33 (0.05)	Mixed sexes
20 May	2.28	0.83	32	0.28 (0.05)	Mixed sexes
27 August	2.74	1.09	17	0.51 (0.05)	Mixed sexes
25 September	3.63	1.13	23	0.46 (0.05)	Mixed sexes
21 October	4.03	1.36	50	0.37 (0.05)	Mixed sexes
22 December	3.07	0.70	34	0.23 (0.05)	Mixed sexes
16 January	3.17	0.92	47	0.26 (0.05)	Mixed sexes

Temperature

Many workers still use surface temperatures as indicative of a species' environment. This is acceptable for running waters, where the temperature profile is uniform, or shallow standing waters where mixing also creates isothermal conditions. Unfortunately the surface temperature is often used to describe deeper waters which can be stratified for much of the year. A misleading impression is created, as the data take no account of the preferred depth of the species or the temperature cycle that occurs there.

Caution is required when relating temperature data to the behaviour

of any species. Loch Lomond can be divided into three sections; a southern, middle and northern area, each with different depth profiles. This creates a situation where different temperature profiles can be recorded in seperate locations within the loch at the same time. The narrow and deep northern region has extensive stratification for most of the year and takes longer to cool in winter than the southern basin. Depths in the southern basin extend to little more than 20 metres, with limited stratification during the summer months (Slack, 1957). The middle section shows intermediate characteristics. The temperature differentials which occur between the different areas of Loch Lomond are at a minimum during February and March when the southern basin is 1 - 2°C cooler than the north.

Temperature profiles were recorded in 50 metres of water within the sampling area at regular intervals for a period of twelve months, and the results are given in table 3. To enable the overall pattern of changes to be seen figure 9 represents the temperature in the range of depths occupied by Coregonus lavaretus. It must be stressed however, that these results relate to one sampling site only, and are not representative of the whole loch.

The thermal characteristics of each region within the loch have been described (Slack, 1957), and because of their relevance to this study are shown in figure 10. The spawning period of Coregonus lavaretus begins in early December, and a comparison of the results for the different regions indicates that the temperature profiles are different at this time.

Light

Loch Lomond is geographically located at latitude 56°N and longitude 5°W. Figure 11 illustrates the photoperiod throughout the year at this location; daylength is given for the following solar altitudes: 0°, -6°, - 12°, and - 18°. Daylength, including the two periods of civil twilight, is defined as the time required for the upper limb of the sun to traverse

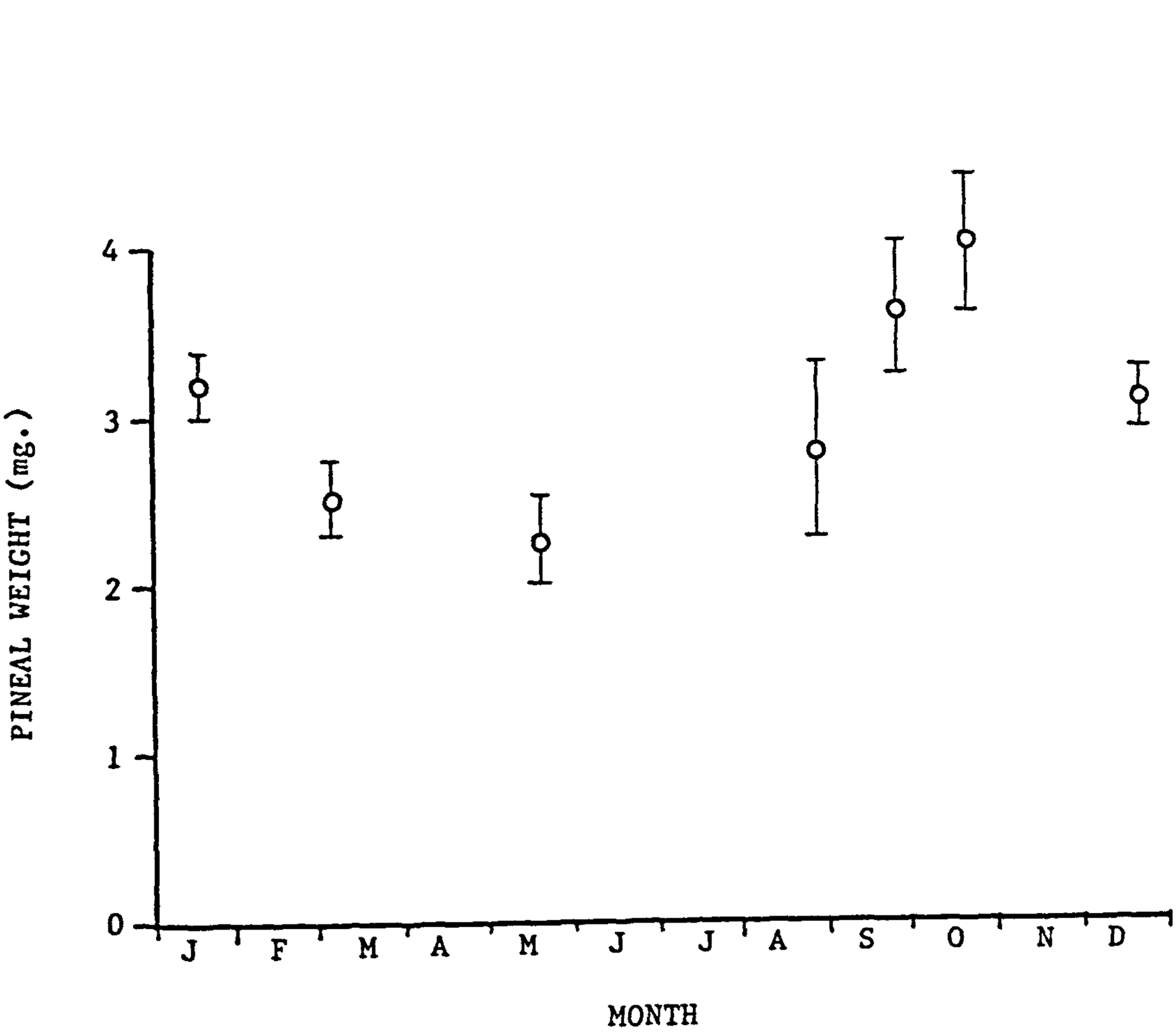
TABLE 3

Monthly Temperature Profiles

Depth (m)	Jan	Feb	Mar	Apl	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
0	5.5	4.4	4.1	6.5	8.0		18.0	18.2	14.2	10.5	8.7	6.7
1	5.5	4.3	4.1	6.5	8.0		17.9	17.5	14.5	10.0	8.7	6.7
2	5.5	4.4	4.1	6.5	8.0		17.2	17.0	14.5	9.8	8.7	6.7
3	5.5	4.4	4.1	6.5	7.8		17.0	17.0	14.5	9.5	8.7	6.7
4	5.5	4.4	4.1	6.5	7.8		16.6	16.7	14.2	9.5	8.7	6.7
5	5.5	4.4	4.1	6.5	7.8		16.6	16.7	14.2	9.5	8.7	6.7
6	5.5	4.4	4.1	6.5	7.8		16.2	16.6	14.2	9.3	8.7	6.7
7	5.5	4.4	4.1	6.5	7.8		16.0	16.4	14.2	9.3	8.7	6.7
8	5.5	4.4	4.1	6.5	7.8		15.5	16.4	14.2	9.2	8.7	6.7
9	5.5	4.4	4.1	6.5	7.8		15.0	16.4	14.2	9.2	8.7	6.7
10	5.5	4.4	4.1	6.5	7.8		14.5	16.4	14.2	9.2	8.7	6.7
11	5.5	4.4	4.1	6.5	7.8		14.0	16.4	14.2	9.1	8.7	6.7
12	5.5	4.4	4.1	6.5	7.8		13.5	16.4	14.2	9.1	8.7	6.7
13	5.5	4.4	4.1	6.5	7.8		13.1	15.4	14.0	9.1	8.7	6.7
14	5.5	4.4	4.1	6.5	7.8		12.6	14.7	13.0	9.1	8.7	6.7
15	5.5	4.4	4.1	6.5	7.8		12.1	13.7	12.5	10.3	8.7	6.7
16	5.5	4.4	4.1	6.5	7.8		11.6	12.5	12.1	10.3	8.7	6.7
17	5.5	4.4	4.1	6.5	7.8		10.5	11.3	11.7	10.8	8.7	6.7
18	5.5	4.4	4.1	6.5	7.8		9.4	10.3	11.5	10.8	8.7	6.7
19	5.5	4.4	4.1	6.5	7.8		8.4	10.0	11.5	10.8	8.7	6.7
20	5.5	4.4	4.1	6.5	7.0		8.0	9.8	11.7	10.9	8.7	6.7
21	5.5	4.4	4.1	6.5	7.0		8.0	9.5	11.0	10.9	8.7	6.7
22	5.5	4.4	4.1	6.5	7.0		8.0	9.2	10.7	10.9	8.7	6.7
23	5.5	4.4	4.1	6.5	7.0		8.0	9.2	10.7	10.9	8.7	6.7
24	5.5	4.4	4.1	6.5	7.0		8.0	9.0	10.7	10.9	8.7	6.7
25	5.5	4.4	4.1	6.5	7.0		7.9	8.7	10.7	10.9	8.7	6.7
26	5.5	4.4	4.1	6.5	7.0		7.8	8.4	10.7	10.9	8.7	6.7
27	5.5	4.4	4.1	6.5	7.0		7.8	8.4	10.7	10.9	8.7	6.7
28	5.5	4.4	4.1	6.5	7.0		7.8	8.4	10.7	10.8	8.7	6.7
29	5.5	4.4	4.1	6.5	7.0		7.8	8.4	10.7	10.8	8.7	6.7
30	5.5	4.4	4.1	6.5	6.9		7.8	8.4	9.5	10.8	8.7	6.7
31	5.5	4.4	4.1	6.5	6.9		7.8	8.4	9.5	10.8	8.7	6.7
32	5.5	4.4	4.1	6.5	6.9		7.8	8.4	9.5	10.8	8.7	6.7
33	5.5	4.4	4.1	6.4	6.8		7.7	8.4	9.0	10.7	8.7	6.7
34	5.5	4.4	4.1	6.4	6.8		7.7	8.4	9.0	10.7	8.7	6.7
35	5.5	4.4	4.1	6.4	6.8		7.7	8.3	8.9	10.6	8.7	6.7

FIgure 8 Mean pineal weight for the period January 1980 to December 80. One SE (0.95) above and below the mean.

Figure 9 The annual temperature cycle over a range of depths in which powan are found.


PINEAL WEIGHT (mg.)
4
3
2
1
0
J
F
M
A
M
J
J
A
S
O
N
D
MONTH

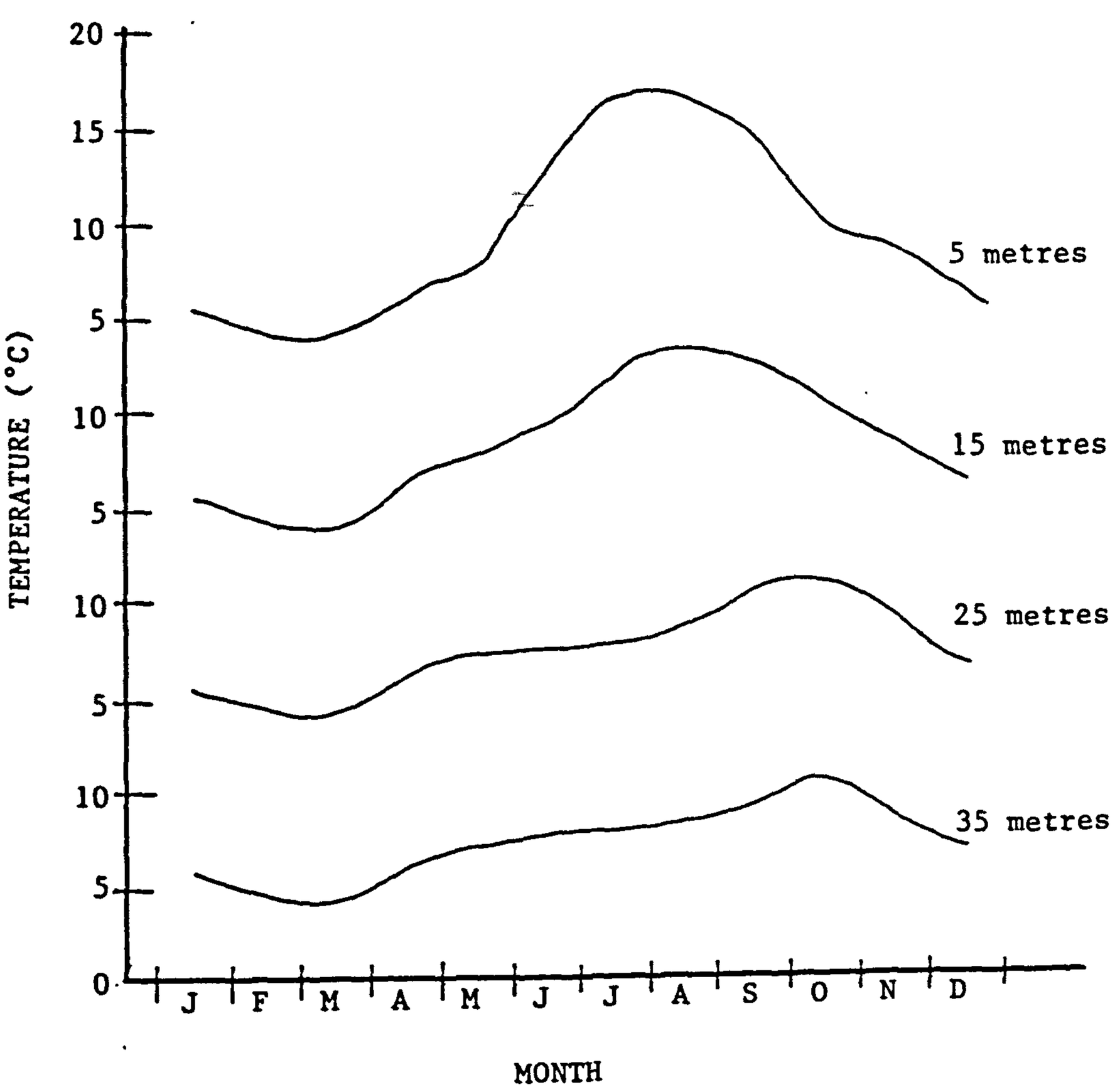

TEMPERATURE (°C)
20
15
10
5
10
5
10
5
0
5 metres
15 metres
25 metres
35 metres
J
F
M
A
M
J
J
A
S
O
N
D
MONTH

FIGURE 10 Isotherms in three areas of Loch Lomond (temperature °C)

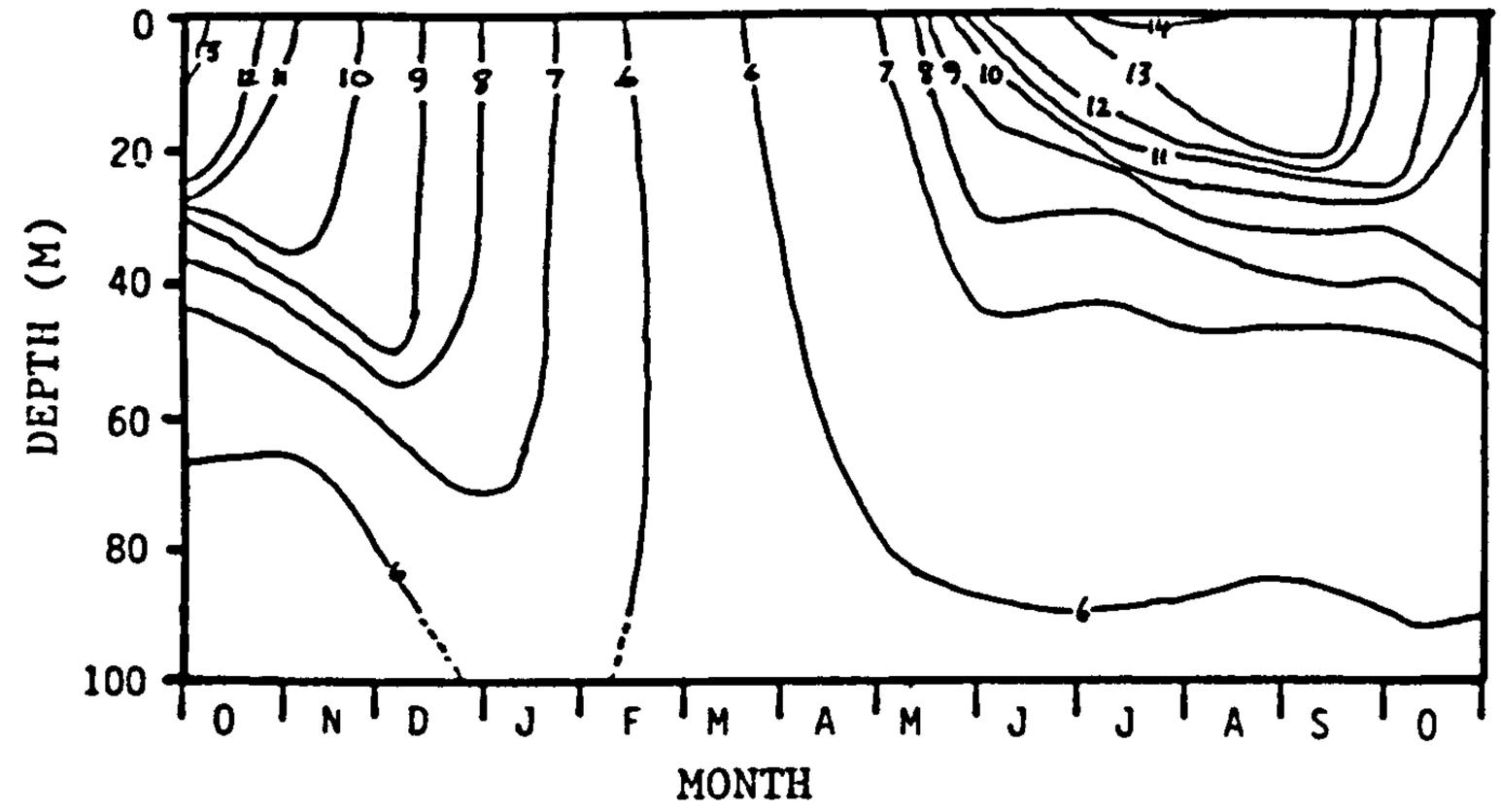
(a) The deep narrow section

(b) The middle section with intermediate depths

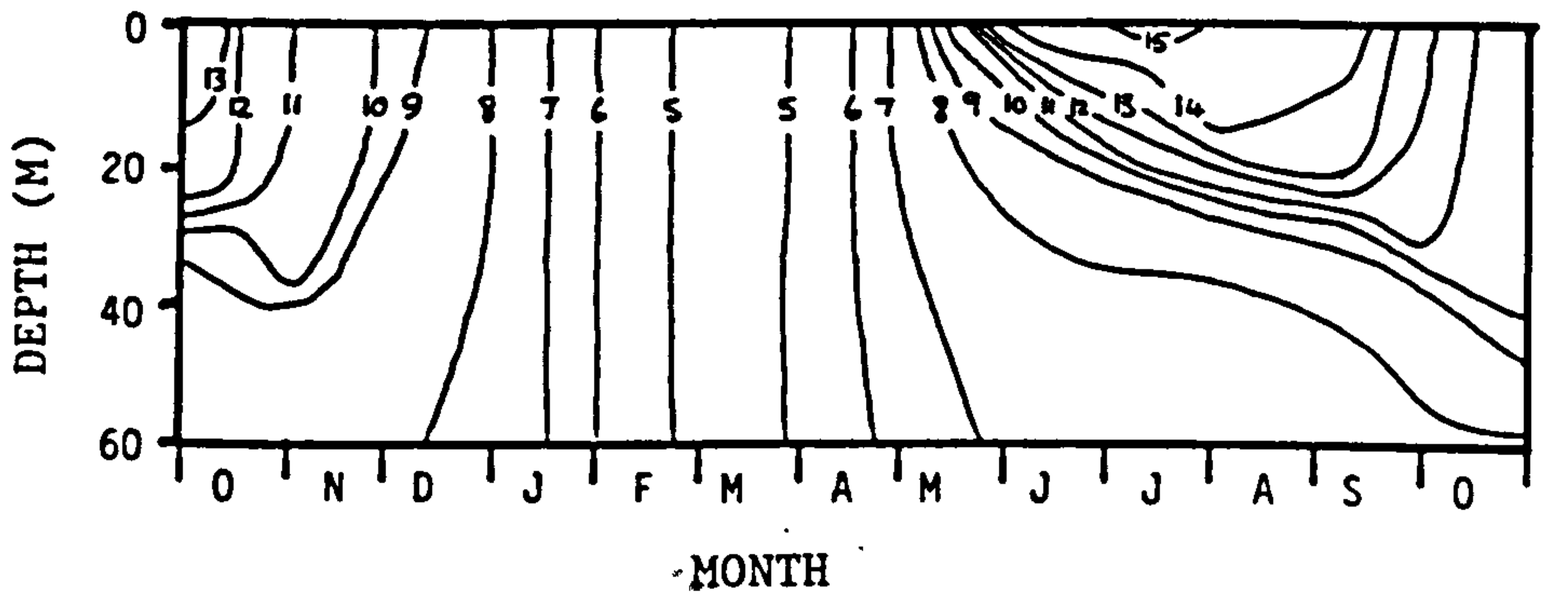
(c) The shallow southern section

(From Slack,1957)

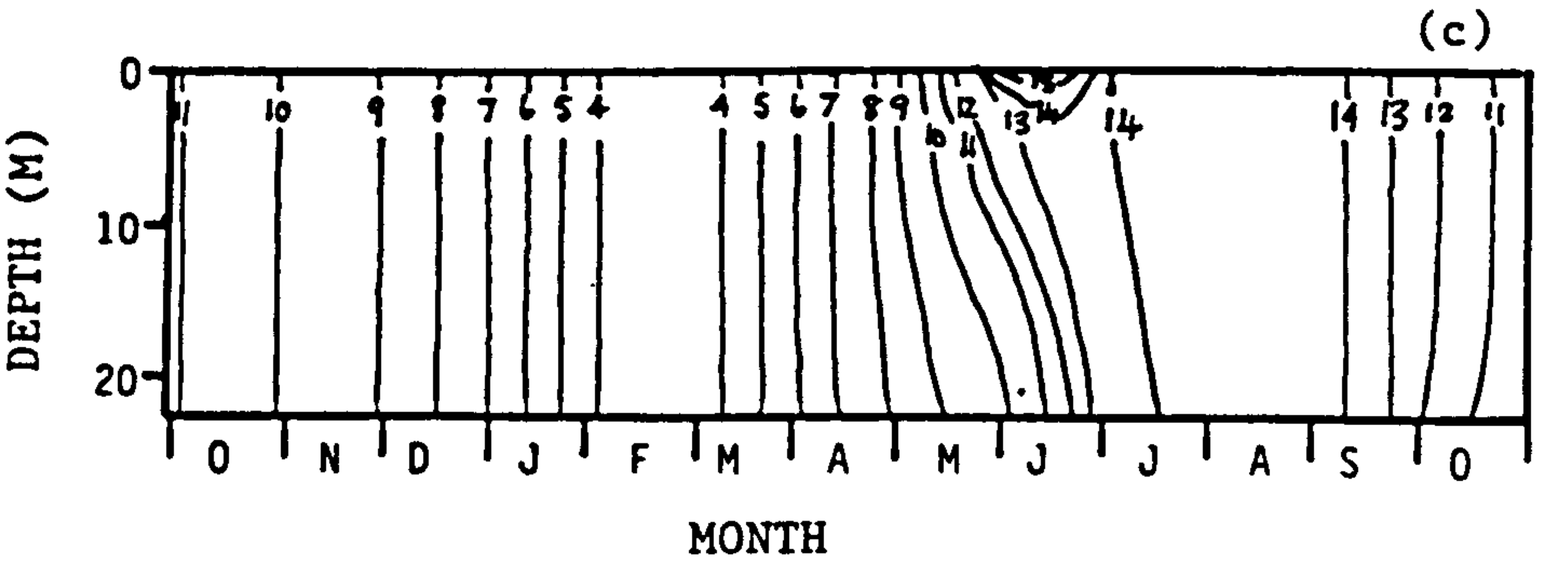
(a)


DEPTH (M)
0
20
40
60
80
100
O N D J F M A M J J A S O
MONTH

(b)


DEPTH (M)
0
20
40
60
O N D J F M A M J J A S O
MONTH

(c)


DEPTH (M)
0
10
20
O N D J F M A M J J A S O
MONTH

an arc from 6° below the horizon in the east to 6° below the horizon in the west. Nautical twilight and astronomical twilight are based on similar calculations, but involve angles of 12° and 18° respectively.

The rate of change in daylength varies according to the solar altitude and season. Table 4 represents in hours and minutes, at solar altitudes of 0°, -6°, -12°, and -18°, the rate of change per week. Variation in rate is similar until after the spring equinox; at 0° the rate of change decreases; at -12° the rate continues to increase to a maximum of x 3.4 (at 27 May); at -18° the rate increases to a maximum of x 3.2 (at 29 April). From 29 April until 10 August there is twilight all night.

Throughout the limnetic zone of freshwater habitats, the various modalities of light (intensity, rate of change, spectral composition, and polarisation) are modified by the time of day, the latitude, and the depth and clarity of the water (Hutchinson, 1975). Additional factors such as the intensity and optical properties of the incident light, which vary dielly and seasonally can be influenced by cloud cover, surface disturbance and atmospheric absorption. The attenuation of light in lakes is the result of two physical processes, absorption and scattering. These factors have a marked effect on light penetration, and vary seasonally.

Another factor which influences the penetration of light and varies seasonally and dielly is reflectance. The reflectance of sunlight from the water surface is essentially a function of solar altitude and can be calculated from Fresnel's law (Hutchinson, 1975). At high solar altitudes, the reflectance of light from a horizontal water surface is only about 2%; it becomes higher at low altitudes, for instance theoretically 35% at 10° altitude, but this effect is reduced by a disturbed water surface. Reflectance may be a significant feature of the environment for Coregonus lavaretus in the approach to spawning. The sun at the winter solstice reaches a maximum altitude of 9.5° at noon and daylength is short; the altitude of the nearest full moon to the solstice will be 53° and therefore

TABLE 4

Information derived from astronomical data for St Andrews, Fife.

Longitude 2.815°W, Latitude 56.337°N.

DATE	SUNRISE 0°	SUNSET 0°	DAYLENGTH	RATE OF CHANGE 0°	RATE OF CHANGE -6°	RATE OF CHANGE -12°	RATE OF CHANGE -18°
17.12	8.32	15.36	6.58	-0.02			
24.12	8.43	15.39	6.56	+0.02			0.00
31.12	8.43	15.45	7.02	0.06			+0.04
7.1	8.40	15.55	7.15	0.13			0.09
14.1	8.35	16.06	7.31	0.16			0.12
21.1	8.26	16.20	7.54	0.23			0.17
28.1	8.14	16.35	8.21	0.27			0.20
4.2	8.01	16.50	8.49	0.28			0.23
11.2	7.47	17.05	9.18	0.29			0.26
18.2	7.31	17.21	9.50	0.32			0.29
25.2	7.14	17.36	10.22	0.32			0.30
4.3	6.56	17.51	10.55	0.33	+0.32	+0.32	+0.33
11.3	6.38	18.06	11.28	0.33	0.32	0.34	0.35
18.3	6.19	18.21	12.02	0.34	0.34	0.34	0.37
25.3	6.01	18.36	12.35	0.33	0.34	0.36	0.41
1.4	5.42	18.50	13.08	0.33	0.34	0.38	0.43
8.4	5.23	19.05	13.42	0.34	0.34	0.39	0.48
15.4	5.05	19.19	14.14	0.32	0.36	0.40	0.55
22.4	4.47	19.34	14.47	0.33	0.35	0.43	1.02
29.4	4.31	19.48	15.17	0.30	0.35	0.46	1.26
6.5	4.15	20.03	15.48	0.31	0.35	0.49	TAN
13.5	4.00	20.16	16.16	0.28	0.35	0.52	TAN
20.5	3.47	20.30	16.43	0.27	0.32	1.00	TAN
27.5	3.36	20.42	17.06	0.23	0.29	1.19	TAN
3.6	3.27	20.52	17.25	0.19	0.26	TAN	TAN
10.6	3.22	21.00	17.38	0.13	0.18	TAN	TAN
17.6	3.19	21.05	17.46	+0.08	0.12	TAN	TAN
24.6	3.20	21.07	17.47	-0.01	+0.03	TAN	TAN
1.7	3.24	21.05	17.41	0.07	-0.10	TAN	TAN

TAN = Twilight all night

FIGURE 11 Photoperiod in hours for latitude 56°N at various solar altitudes.

(a) Solar day, 0°

(b) Civil twilight, -6°

(c) Nautical twilight, -12°

(d) Astronomical twilight, -18°

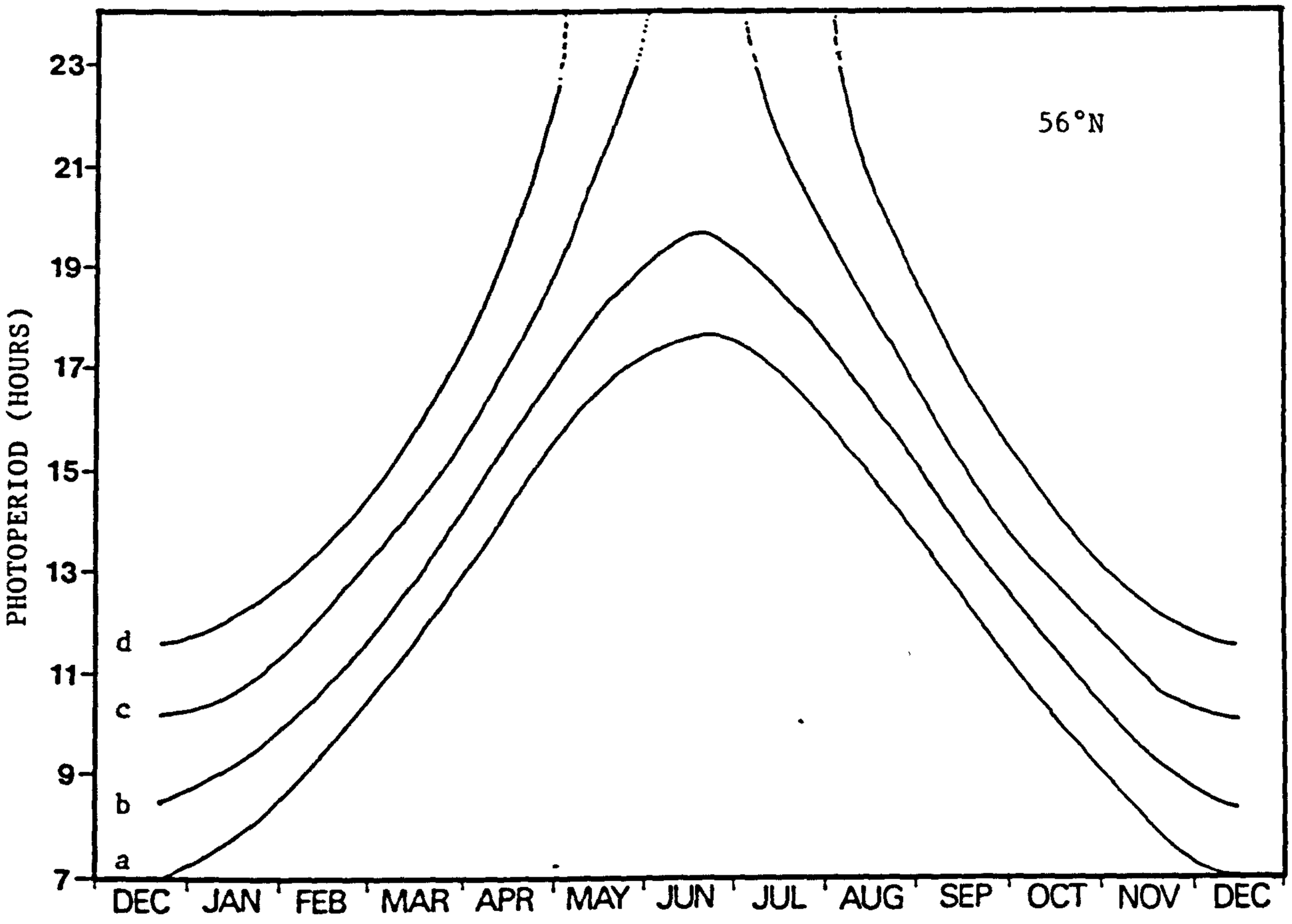
FIGURE 12 Semi-log plot of depth (metres) against the percentage transmission of green light (504nm).

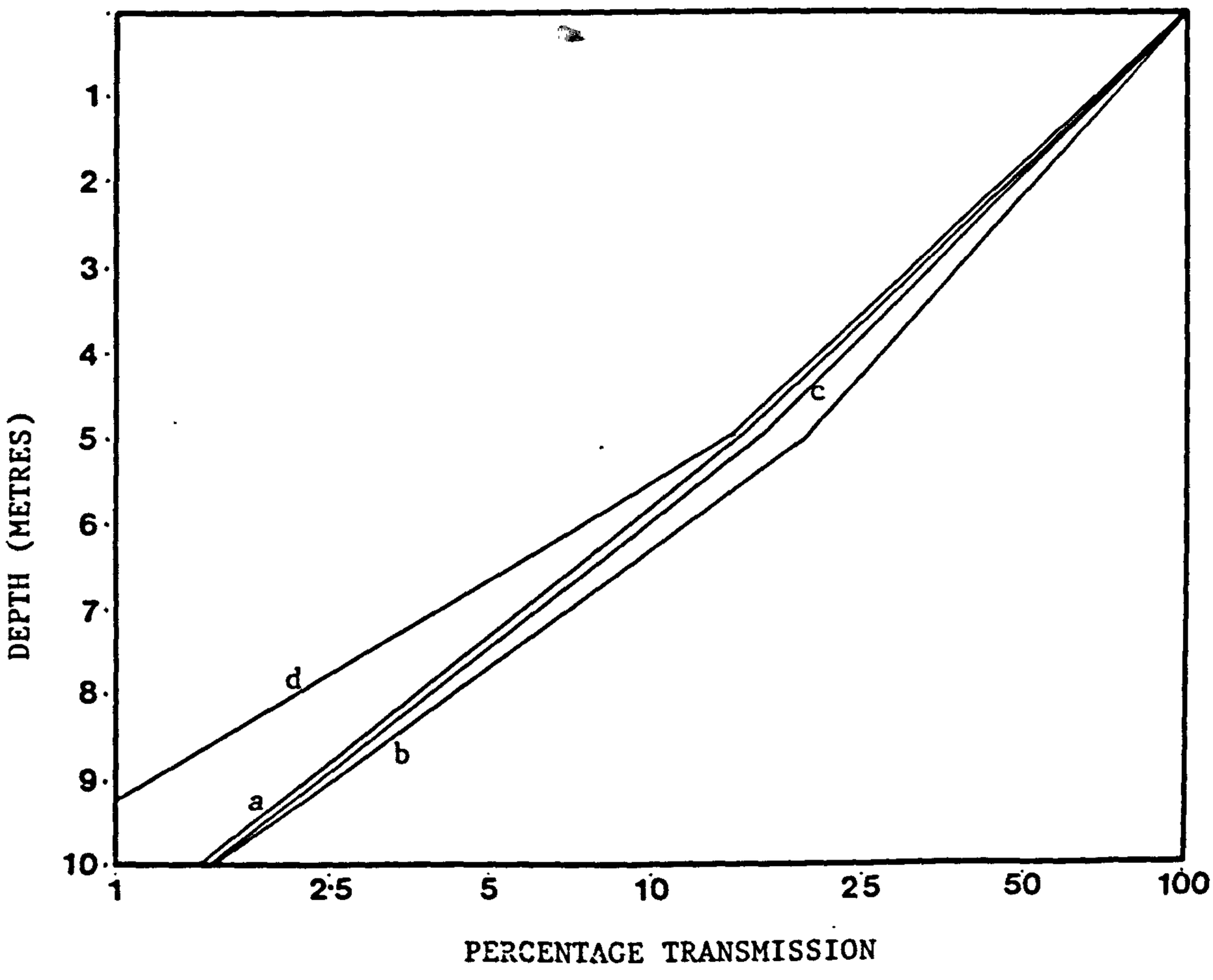
(a) = September, 1.5% at 10 metres

(b) = October, 1.5% at 10 metres

(c) = November, 1.57% at 10 metres

(d) = December, 0.6% at 10 metres


56°N
PHOTOPERIOD (HOURS)
23
21
19
17
15
13
11
9
7
d
c
b
a
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
MONTH


DEPTH (METRES)
1
2
3
4
5
6
7
8
9
10
c
d
b
a
1
2·5
5
10
25
50
100
PERCENTAGE TRANSMISSION

penetration through the water surface will be high.

Methods for measuring light in the aquatic environment are not all uniform which can lead to problems in interpreting data. Some workers measure light in luminous watts per unit area squared which gives rise to units like metre candles (mc) and lux. Luminous watts are based upon the integration of the total energy flux, over the range of visible wavelengths. Other workers measure underwater light in radiant watts/unit area over either a unit solid angle (radiance) or from all angles (irradiance), (Segal, 1970). The difficulty is that both groups are not measuring the same thing, and attempts to convert from one to the other is not possible. Furthermore, the units employed have other shortcomings in that the photometer used in an investigation may have a spectral response which is different from that of the experimental animal; and light may be measured which is not of functional significance.

The results are presented in the form of a semi-log plot of percentage transmission against depth for the top 10 metres. During the period from September to December the intensity of green light (504nm) at 10 metres depth varied between 0.6% and 1.6% of the surface value (Fig. 12). Within the first metre depth, light intensity had dropped to 70% of the surface value. As a comparison, transmission of green light in Loch Uanagen fell to 6% of the surface value at 10 metres (Spence et al, 1971).

Lunar photoperiod

The sidereal month is the time taken by the moon to circle the earth and return to the same position with respect to the stars. Figure 13 shows the meridian altitude of the moon on each day throughout the year and the period between successive peaks and troughs is equal to 27.32 days. The period between new or full moons is equal to 29.53 days and represents the synodical month (lunation); which is out of phase with the sidereal month. The effect is to create a cycle of full moon meridian altitudes (Fig. 13) which is maintained from year to year. The altitudes given are specific for

latitude 56°N. At a higher latitude the peak altitude would be lower and towards the equator it would be higher.

The phase represents the area of the moon circle which is reflecting light on a scale from 0 to 1 (Fig. 14). For 16 days of the synodical month the phase is greater than 0.5 and for 11 days it is greater than 0.8. Moonlight could therefore represent a significant feature of the environment for animals with the sensory apparatus to detect it.

The meridian altitude of the sun is at its lowest (<10°), and daylength is shortest at the winter solstice. At this time the meridian altitude of the full moon is at its highest (53°) and moonlight is available throughout the long winter night. Figure 15 compares the relative amounts of moonlight at the time of the full moon from May to December. If low light intensities are significant to an animal, the full moon period could represent several days of continuous light; which would be independant of season.

The fact that the altitude of the full moon during December to January is beyond 50°, indicates that reflectance will be less than 3%. Penetration through the water surface will therefore be at a maximum for the year.

The intensity of moonlight at the period of full moon was found to be rarely greater than about 0.7 lux (Saunders, 1977). A lower value of 0.25 lux was reported by Blaxter and Holliday (1958). The fact that the measurements are in luminous watts, means that they are based on the integration of the total energy flux, and represent the range of visible wavelengths. Comparison is handicapped because neither author mentions the spectral sensitivity of their photometer or indicates when the readings were taken. The light intensities experienced during the twilight period range between 10^{-1} - 10^{-2} lux (Segal, 1970). It seems probable therefore, that the intensity of moonlight within the surface metre would fall within this range.

Water Level

The water level during the approach to spawning could rise or drop by as much as 1 metre (Figs. 16,17). Records for other years indicated a range of 1.5 metres during December. The water level during December 79 dropped to 7 metres above chart datum and in 1980 the loch was higher at 9.1 metres when spawning began. The Isoetes zone extended from 3 metres depth on gently sloping bottoms and might be a spawning substrate. Reductions of 1.5 metres would substantially reduce the area available for spawning. Although there was daily variation in the level of Loch Lomond, the mean December value was between 8 to 9 metres above chart datum (Figs. 16,17). If spawning occurs in areas of wave-washed gravel it may be significant that the water level can drop considerably in the early part of the year; ova would be stranded above the water line. No correlation was observed between loch level and the spawning period.

FIGURE 13 The meridian altitude of the moon for each day throughout the year. The period between successive peaks and troughs is equal to 27.32 days (siderial month).
The period between new or full moons is equal to 29.53 days and represents the synodical month (lunation).

O = meridian altitude of the full moon (1979).

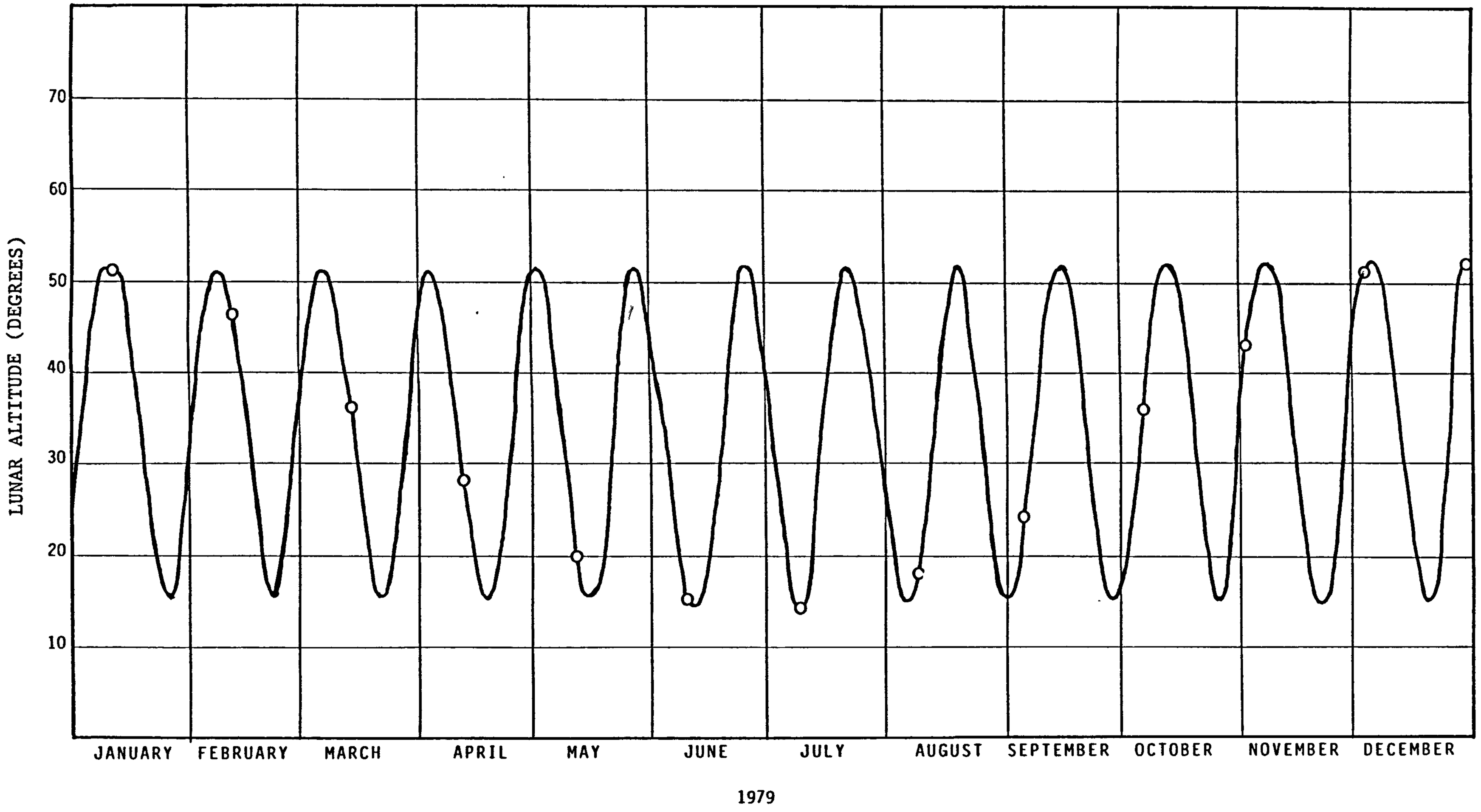

LUNAR ALTITUDE (DEGREES)
70
60
50
40
30
20
10
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER
1979

FIGURE 14 The area of the moon circle (phase) which is reflecting light on a scale from 0 (new moon) to 1 (full moon). The phase is shown for each day of the synodical month.

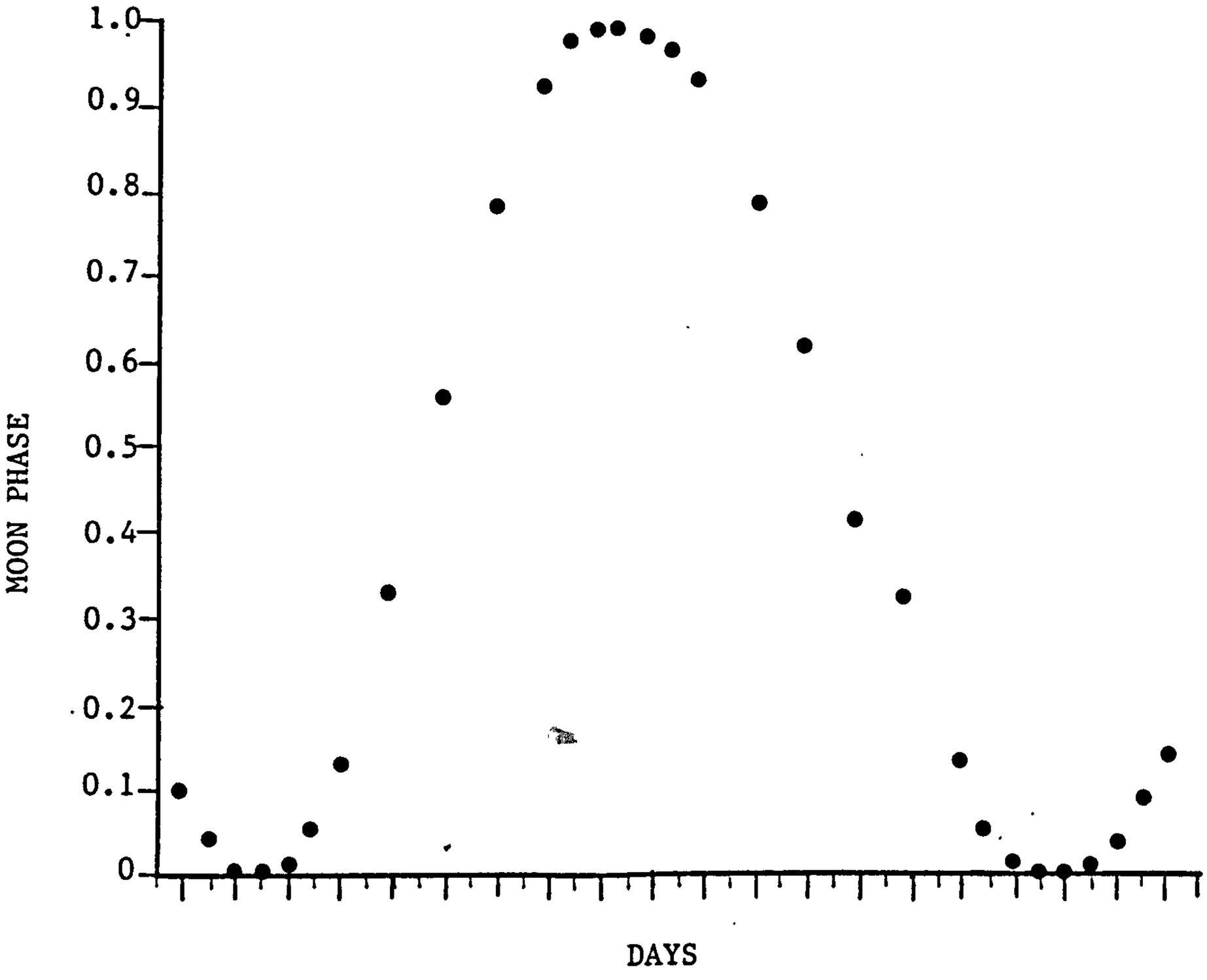

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
MOON PHASE
DAYS

FIGURE 15 The meridian altitude of the full moon from May to December 1979. This diagram indicates the relative amounts of moonlight at the time of the full moon.

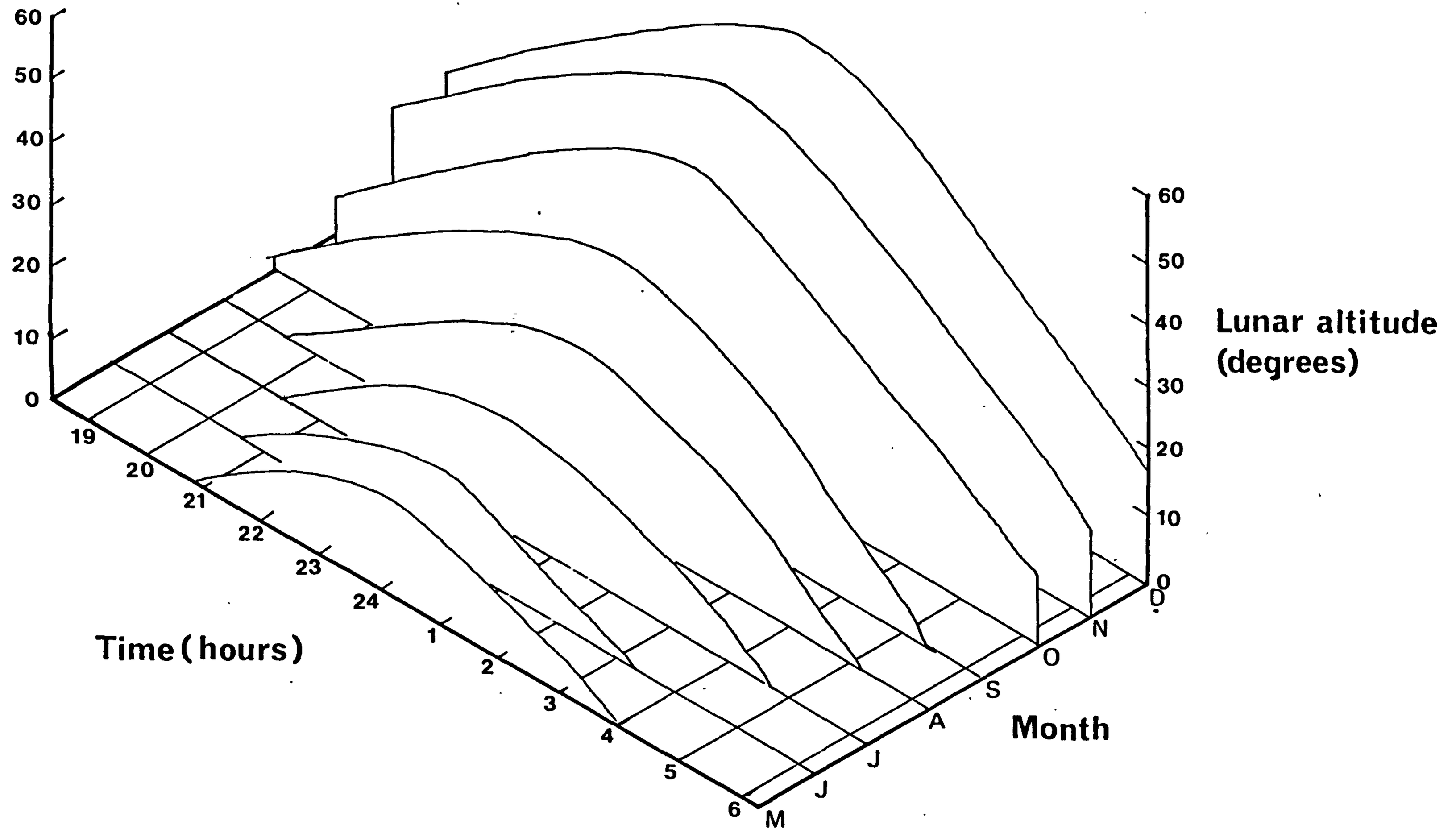

60
50
40
30
20
10
0
19
20
21
22
23
24
1
2
3
4
5
6
Time (hours)
M
J
J
A
S
O
N
D
Month
60
50
40
30
20
10
0
Lunar altitude
(degrees)

FIGURE 16 The surface level of Loch Lomond in metres above chart datum for the period before the onset of spawning. Values for every third day

(a) 1979

(b) 1980

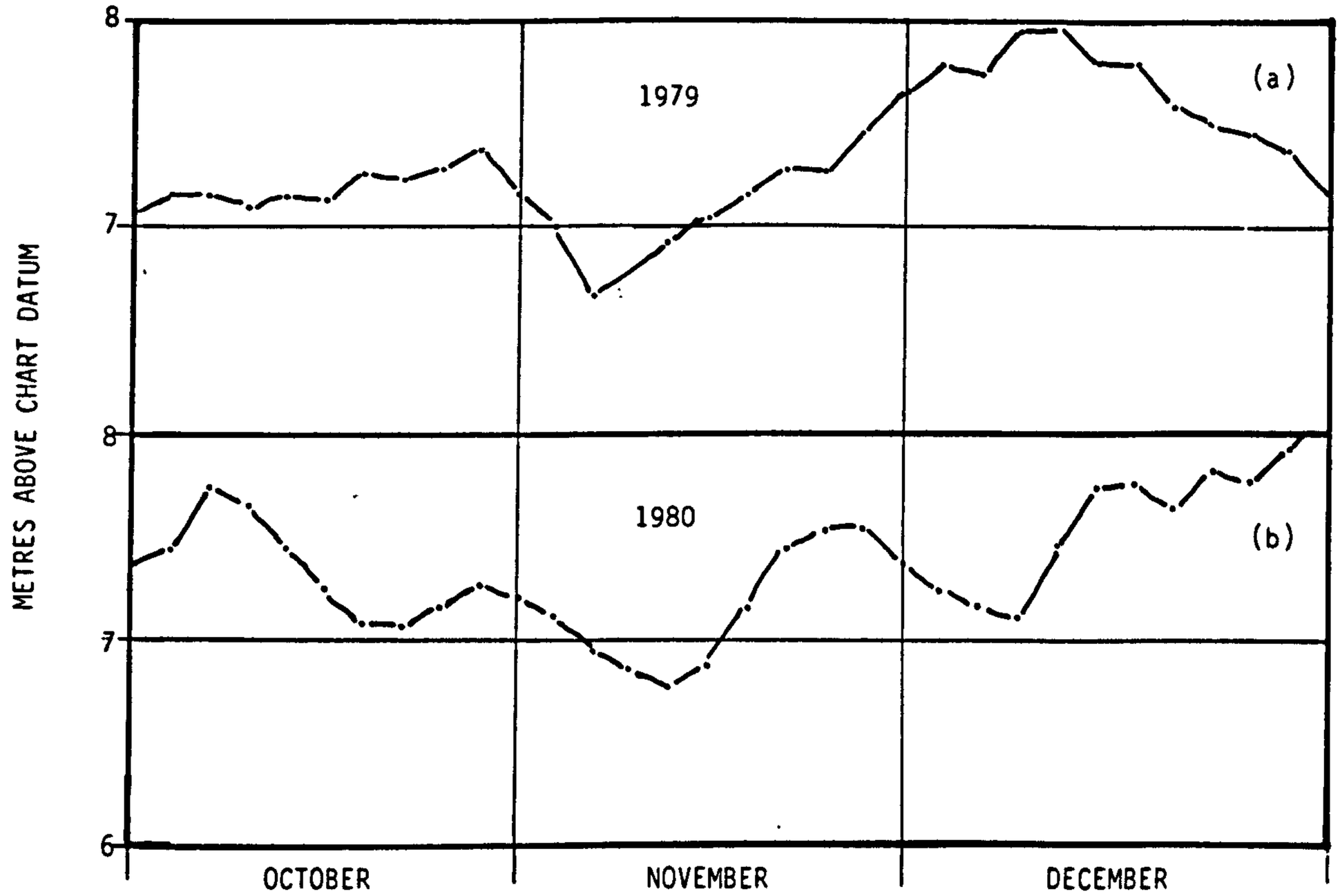
(Clyde River Purification Board)

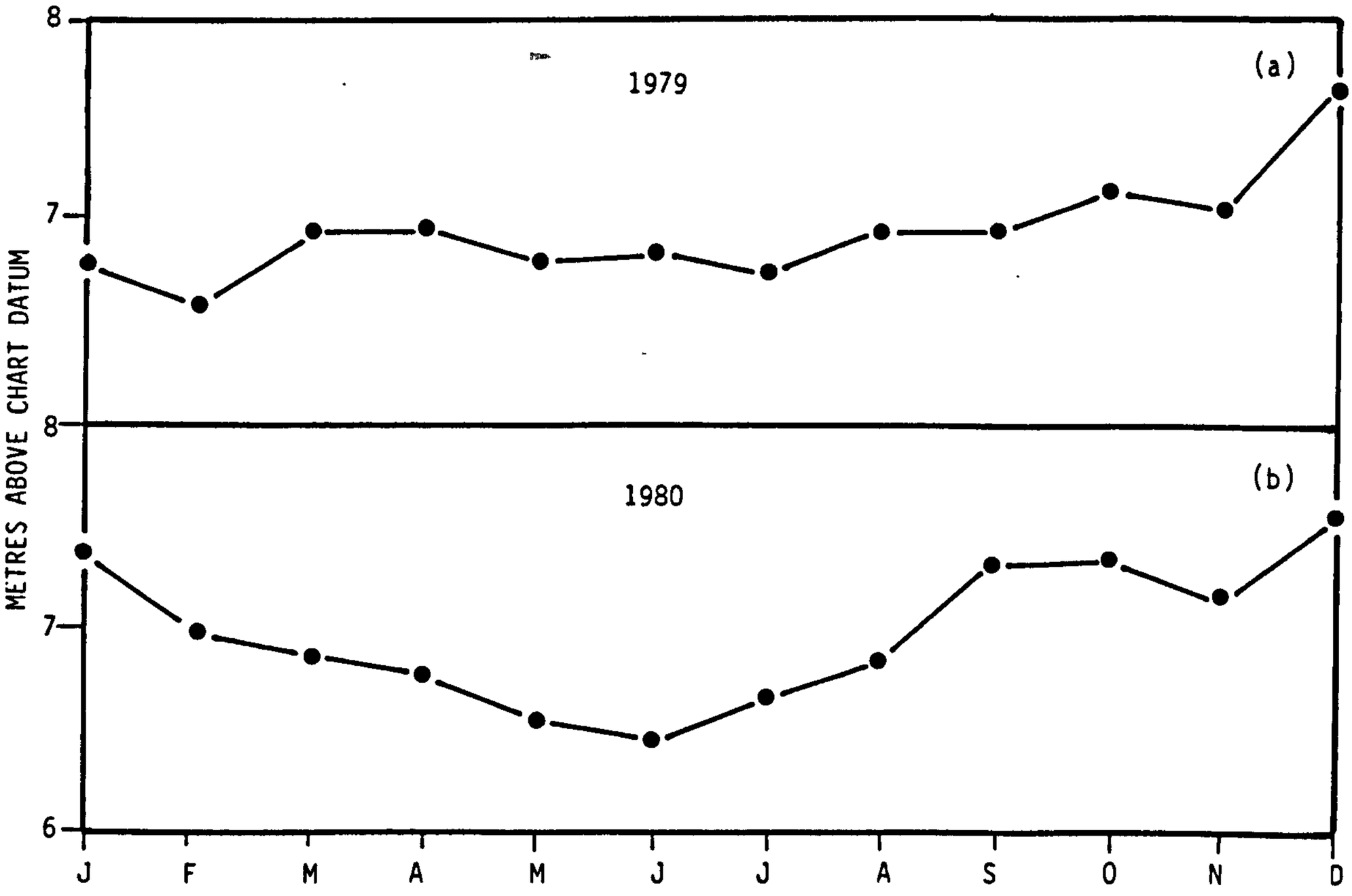
FIGURE 17 The mean surface level of Loch Lomond in metres above chart datum

(a) 1979

(b) 1980

(Clyde River Purification Board)


8
7
8
7
6
METRES ABOVE CHART DATUM
1979
(a)
1980
(b)
OCTOBER
NOVEMBER
DECEMBER


8
7
8
7
6
METRES ABOVE CHART DATUM
1979
(a)
1980
(b)
J
F
M
A
M
J
J
A
S
O
N
D

Discussion

The spawning period of Coregonus lavaretus can apparently begin at any time from early December to early January. The period of around 30 days suggests that the mean date for the start of spawning is 23 December with a standard deviation of about 9 days. Cushing (1975) compared the mean date of peak spawning in several temperate zone marine teleosts and the Pacific sockeye salmon, and found the standard deviation was about one week. The variation shown in the date of the beginning of spawning by Coregonus lavaretus is also found in European coregonid populations (Zuromska, 1982). The reason why whitefish and vendace show such a large spread in spawning times is not clear, especially as the duration of spawning is so short.

The environment occupied by Coregonus lavaretus at this time is essentially stable with temperature approaching homothermy and photoperiod at the winter solstice. The fish spawn on suitable substrates throughout Loch Lomond (Maitland, 1968) yet the temperature between different regions may differ by 1 to 2°C at this time. The initiation of spawning in the population appears to be precise and sudden, yet there is no evidence of any correlation with a specific temperature. European whitefish populations begin spawning within the range 2.5° to 8°C, and at different temperatures in different years; 8°C seems to be the maximum temperature at which spawning will begin (Zuromska, 1982). This may be due to the fact that coregonid eggs suffer high mortalities at and above this temperature (Bagenal, 1970). The cooling of the loch is obviously a seasonal event, but the rate of change in temperature is very slow, and the notion that it is the approach of homothermy which induces spawning (Slack et al, 1957; Zuromska, 1982) is not compatible with the precise timing of the event. Temperature is probably very important as a secondary factor in the timing of final maturation and ovulation, and it may be possible to delay these processes by artificially maintaining temperature above 8°C in an

experimental situation.

Maitland (1968) has suggested that the start of increasing daylength after the winter solstice may act as a cue to initiate spawning in the population. The rate of change in photoperiod during the weeks approaching and after the solstice is very slow and unlikely to represent a cue. Moreover, during this study spent males and females were recovered both before and at the solstice on consecutive years.

Water level has also been postulated as a contributory factor in the initiation of spawning, as it is usually high at the time of spawning and therefore covers suitable spawning substrate (Maitland, 1968). An analysis of water level in Loch Lomond during the approach to and during the spawning period over several years has revealed that major changes in water level occur under the control of the water authority. No correlation was found between loch level and spawning date.

The photoperiod is dictated by the latitude of the loch, and daylength follows a seasonal cycle. Conventionally, photoperiod is expressed as the interval between sunrise and sunset at a solar altitude of 0°. It is commonly assumed that at the equinoxes, all parts of the globe experience a 12 hour day and a 12 hour night (12L/12D). However, if daylength is measured by an animal through a photoreceptive transduction system which responds to low light intensities, the twilight period is also significant. At the equinox (56°N), daylength can vary between 12 and 16.7 hours depending on the threshold of sensitivity of the animals photoreceptors. Salmonid reproductive cycles are known to be regulated by photoperiod (de Vlaming, 1974) but the nature of the cues remain unclear. Recently, the twilight periods were shown to be important in the entrainment of endogenous circadian rhythms in a teleost Couesius plumbeus, and to effect the length of the circadian period [τ] (Kavaliers & Ross, 1980). It is difficult therefore to relate a fixed photoperiod to the seasonally varying natural photoperiod without (a) an understanding of the behaviour

of the animal (b) knowing the sensitivity of the animal's transduction system.

A system designed to measure daylength must be accurate and therefore it seems unlikely that daylength as defined by a solar altitude of 0° would be used. The system should try to minimise 'noise' as much as possible, and the twilight periods after -6° are attractive in this respect. The effect of weather and cloud on the low light intensities are minimal at this time (Hughes et al, 1984), and the light levels are unlikely to be duplicated during the period between sunrise and sunset. The use of a transduction system which is sensitive to the lowest light levels is most likely to receive a pure signal and result in the greatest accuracy. Failure to mature in synchrony with the rest of the breeding population is under severe selection pressure. It is to be expected therefore, that a species will have evolved a sensory system adapted to the most reliable or noise-free zeitgeber.

The only major environmental variable at the time of spawning is the lunar cycle. Although an effect of lunar phases is known in teleosts: the grunion Leuresthes tenuis (Ayres) (Walker, 1949), and other marine fish (Lowe-McConnell, 1979) it is not clear whether the fish respond to the effect of tides or the lunar photoperiod. An effect of lunar phases on the timing of reproduction is known in amphibians (Schwassmann, 1971) and mammals (Sinclair, 1977). The intensity of full moon is 0.7mc (metre candles) which is within the range of light intensities experienced during the twilight .1 to 0.001mc. It is also within the sensory competence of some teleosts (Blaxter, 1970).

The phase differences between the sidereal and synodical months cause the timing of the full and new moons to differ from year to year. The evidence suggests a relationship between the full moon phase of the lunar cycle and the onset of spawning; these results require to be verified experimentally before concluding that moonlight is used by Coregonus

lavaretus to cue spawning activity.

The environment occupied by Coregonus lavaretus follows a regular pattern which varies seasonally and is repeated annually. Temperature in the loch changes according to season but follows an annual cycle which varies according to depth. Unlike photoperiod which follows a precise cycle each year, the timing of the temperature cycle is influenced by the weather, and does show annual variation. The concept of a 'preferred' temperature range is questionable as the species is known to undergo extensive vertical migrations through the water layer. It is more likely that their spatial distribution is linked to feeding, which is predominately pelagic during the summer and benthic in winter (Slack et al, 1957).

It has been reported for powan (Maitland, 1968) and other coregonids (Dembinski, 1971; Haram, 1968) that shoaling occurs during the day in the depth range 20 to 30 metres. There may be a relationship therefore between the temperature cycle at those depths, which peak during September,and the rapid rate of increase in gonadosomatic index which occurs at the same time.

The period of homothermy lasts until the end of April when stratification begins in the mid and northern sections of Loch Lomond. The 8°C isotherm appears during May in all regions of the loch and lasts until December when the period of homothermy begins (Slack et al, 1957). It may be therefore that temperature does influence the timing or progress of recrudesence, either as a primary or secondary factor. So far the interaction of temperature and photoperiod on the regulation of salmonid reproductive cycles has remained largely unexplored (Scott & Sumpter, 1983).

Rainbow trout Salmo gairdneri, take 50 degree (°C) days from the resumption of meiosis (final maturation) to ovulation (Bry, 1981). If this is a typical figure for salmonids, it would mean that at lake temperatures of 6° to 8°C, ovulation would occur after 8 to 6 days respectively in Coregonus lavaretus. It may be therefore, that females do remain near the

surface as postulated by Fuller et al (1976), where final maturation is induced by moonlight; as ovulation approaches the females migrate to the spawning grounds.

The reproductive cycle as indicated by gonad weight and gonadosomatic index is rigidly ordered, and follows a similar pattern each year. The condition factor remains at its lowest level until May each year. How much longer it remains low was not established, but a rapid increase in condition towards some limit occurs by the end of July. This is a fixed feature of powan physiology which occurs at the same time each year and is related to the intensive feeding at this time. It is during the period when the condition factor is increasing that the gonadosomatic index of both males and females begins to rise. Rashid (1984) has shown that exogenous vitellogenesis begins during July, and secondary spermatocyte development begins at the same time (Fuller et al, 1976).

Although of limited relevance to teleosts, an effect of minimum body weight linked to a critical fat level on the onset and maintenance of menstrual function in human females has been shown (Frisch, 1977). The hypothesis of Reshetnikov et al (1970), that the onset of sexual maturity in Coregonus lavaretus is associated not only with the attainment of definite size and age, but also with a certain level of body fat is attractive. Such a system might provide a threshold condition which all individuals in the breeding population would have to reach before beginning the most demanding stage of the reproductive cycle. The relationship between body fat levels, the increase in condition factor, and the onset of exogenous vitellogenesis and secondary spermatocyte development requires to be investigated experimentally in this species.

CHAPTER 1

The Reproductive Cycle

Summary

The reproductive cycle of the powan Coregonus lavaretus (L.) is rigidly ordered and follows a similar pattern annually. Individual physiological phases occur at the same time each year.

Endogenous vitellogenesis begins during April as loch temperatures begin to rise and the rate of change in daylength at negative solar altitudes approach a maximum value.

Exogenous vitellogenesis begins throughout the female breeding population in July during a precisely timed (annually repeated) rise in both condition factor and somatic condition factor. Secondary spermatocytes and spermatids begin to appear in the male breeding population at the same time.

The mean weight of the pineal organ in adult fish varies between 2.28mg in May to 4.03mg during October. There is no indication of any significant variation in pineal weight between sexes. There is some suggestion of a correlation between body weight and pineal weight.

The development of embryos is temperature sensitive and must occur at <9°C. Hatching occurs in the spring, over 3 months after spawning, and its timing is critically important. The fish must therefore predict when conditions will be suitable.

Photoperiod and loch temperature at the time of spawning change very little and are unlikely to act as proximate cues. Water level varies randomly and is not correlated with spawning time. The synodical cycle of full moons is the only major environmental variable at this time. The results suggest that the full moon may act as a proximate cue stimulating the onset of final maturation, ovulation and spawning. Entrainment to a lunar cycle would enable the population to predict time accurately and provide a suitable proximate cue to enable spawning synchrony within the breeding population.

CHAPTER 2

Echosounding Survey

Introduction

Information on the spatial and temporal distribution of teleosts is very limited, and largely concerned with commercially important species, mainly marine. References to freshwater species are largely based on anecdote; however, the coregonines have received some attention with several echosounding studies (Haram, 1968; Maitland,1967a,1968; Dembiński, 1971). References to the behaviour of the Loch Lomond powan Coregonus lavaretus are: Brown (1891), Service (1906), Slack et al (1957), Maitland (1968) and Scott (1979).

Technical aspects of echosounding are adequately dealt with in a number of publications: sonar equation (Craig, 1973), theory relative to fisheries work (Tucker, 1967), practical problems (Natarajan et al,1980), basic principles, method and survey design (Suomala & Yudanov, 1980). The principle of operation of an echosounder may be summarised as follows. It functions by transmitting pulses of acoustic energy, and recording the returning echoes. The time that has elapsed between the transmission of the pulse and the reception of the echo, is a measure of the range of the object.

In the past workers have used low frequency, wide beamwidth (30°) transducers which were inefficient at detecting fish very near the bottom; the large areas covered by the beam lowered the resolving power of the equipment. Low scan rates (60 to 120 soundings/minute) and compressed scales (0 to 110 metres), further handicapped interpretation. Narrow beamwidth (10°), high frequency echosounders which are now available offer the possibility of high resolution work. The new equipment has increased sensitivity (scan rates >500 soundings/minute) and resolution, but suffers from limited range; absorbtion loss in water increases rapidly as frequency increases (Tucker, 1967). However, the limited range has no significance

for this study where the depths are relatively shallow.

A possible relationship between the onset of the full moon and the time of spawning has been postulated (Scott, 1979). The attenuation of light in freshwater is considerable and the intensity of moonlight is low. If powan do monitor the cycle of full moons it would be logical to expect them to spend time in the surface layer during the night where the effect of attenuation would be minimal. Only very limited information exists on the distribution of Coregonus lavaretus during the night (Maitland, 1968)

The twilight periods, when used by organisms to measure daylength, can offer considerable accuracy (Hughes et al, 1984). Electrophysiological experiments on teleost pineals have suggested a photoreceptive role which may be adapted to the detection of low light levels associated with the twilight periods (Hanyu et al, 1977; Falcón & Meissl, 1981). Unfortunately no reference is made in these papers to the behaviour of the experimental animal in its natural environment. If fish are to measure daylength by monitoring the the twilight periods it seems reasonable to assume that there may be a behavioural adaptation which would enable them to do this. Coregonines are known to migrate to and from the surface during the twilight periods but precise details of the movements are lacking.

The aim of this investigation was to obtain qualitative data on the spatial and temporal distribution of Coregonus lavaretus in Loch Lomond. In particular an attempt was made to confirm existing ideas on the distribution at spawning, to define more precisely the twilight migrations and the movements of the fish during the night.

Materials and Methods

Loch Lomond is 36.4km in length and has a surface area of 71.1km^2 (Shafi & Maitland, 1971). The size created problems not found on other surveys where smaller surface areas enabled fish to be located relatively easily and cross sectional transects were possible. To minimise the practical problems, surveys were restricted to within 2.5km radius of the University Field Station, Rowardennan . Fortunately the spawning grounds are reasonably well defined, and in relatively shallow water, so the task of locating the fish was simplified. The diel migration of the powan from offshore banks and ridges also allowed the fish to be traced with relative ease.

The practical problems associated with an echosounding survey are considerable, especially with the resources which were available. On many occasions the survey work had to be aborted due to bad weather, as surface conditions were unsuitable for echosounding. Plate 1 shows the survey area and the exposed position of the Ross Islands.

Investigations were carried out with a Furuno echosounder: type FG 200 Mk.3, 200 KHz frequency, 10 watts acoustic power, Four separate scales were available: 0 - 22m, 20 - 42m, 40 - 62m, 60 - 82m. The echogram was recorded electrostatically on dry paper which advanced at a fixed rate of 17mm per minute. The sounding rate was 511 pulses per minute (8.5 pulses/ sec.), and the pulse duration was 1.0 milliseconds.

The echosounder was a portable instrument, and the transducer was secured (temporarily) to the boat in such a way that the acoustic beam was transmitted vertically. The transducer had a beam width of 10°(3 dB). The beam width is the angle between two points in a power polar diagram where the intensity falls to half of its maximum value (3 dB), and is therefore a measure of the directivity of the transducer. An acoustic beam is not simply a cone with well defined boundaries; it is a pulse of acoustic energy which is greatest on the central axis of the beam, but which

diminishes to zero intensity as the angle from the main axis increases. Other minor or side lobe beams are produced, which can generate significant interference over steeply sloping bottoms.

A feature of the echosounder was that no information could be recorded in the 3 metres immediately below the transducer. In order to prevent direct feedback of the transmitted signal no echoes are recorded for a short time after transmission, which creates the blank zone immediately below the surface on echograms (Haram, 1975). This feature only applies to recordings in the depth range 0 to 22 metres. The acoustic reflective capacity of fish is attributed in the main to the swim bladder acting as an air water interface; other components of the body such as flesh and vertebral column also contribute to the echo (Suomala & Yudanov, 1980).

Features of the freshwater environment such as detritus, gas bubbles and the thermocline can also give rise to echoes which may be difficult to differentiate from those of fish. Echo recordings produced by smaller organisms such as fish larvae and plankton appear as fine grained clouds usually over large areas. Their identification is possible however, as the character of these traces does not alter with any change in vessel speed (Natarajan et al, 1980).

The appearance of the echogram can reveal information about the nature of the lake bed. Hard bottoms such as rock reflect the acoustic beam towards the surface which in turn reflects the echo. This continues until the acoustic energy is finally dissipated. The sound is reflected back to register a trace at exactly twice or three times the depth, which gives rise to multiple bottom echoes. Shoals of fish which are close to the bottom can also give rise to multiple echoes (Haram, 1965).

Individual fish are detectable when they are separated from one another and from other targets by at least one pulse length in depth and at least one beamwidth horizontally (Tucker, 1967). Pulse length in the water is defined as the distance between the leading edge and trailing

edge of the pulse. The duration of the pulse is 1 millisecond (T), and the velocity of propagation of sound in freshwater was taken to be 1500 metres/second (c); the pulse length (L) is equal to cT which for the Furuno is 1.5 metres. When the density of fish exceeds the resolving power of the echosounder, the recording takes the form of a multiple echo trace, or as a distinct solid trace. The distinguishing feature of the layer type traces produced by fish is the presence of single fish traces at the upper and lower borders of the layer (Natarajan et al, 1980).

To facilitate recognition of fish echoes which are within a pulse length of the bottom, the 'white line' device is available on the echosounder. When the powerful bottom echo is received at the transducer a bias voltage is applied to the recording stylus, preventing it marking for a few milliseconds. A blank area is therefore created under the bottom profile. The relatively weak fish echoes do not trigger the bias voltage and are recorded normally (Tucker, 1967).

A fish returns a varying amout of echo intensity, depending on its target strength (Suomala & Yudanov, 1980), which has to be greater than the background noise for detection to occur. Obviously the deeper the fish the weaker will be its echo; fish at the periphery of the acoustic beam will give a weaker echo than those on the central axis. Fortunately, the depths searched during this study were relatively shallow, and therefore the intensity of the returning fish echo was within the sensitivity of the transducer.

There is,at present, a lack of standardisation in calibration measuring procedures. The use of 'ping-pong' balls as standard targets (Haram, 1968) for the calibration of transducer response is in doubt, and solid metal spheres are now considered to possess more stable properties (MacLennan, 1981); there is no currently accepted standard target (Suomala & Yudanov, 1980). The objective of this study was not quantitative and calibration of the equipment was thought unnecessary. Depths were

checked by shot line, and the nature of the lake bed was examined by skin diving.

The interpretation of echograms is largely subjective and absolute certainty as to the identity of fish traces is not possible. However, the experience gained from netting over four years; direct observation by skin diving; and knowledge of other species in the area all promote confidence in the results. Whenever possible the identity of the fish traces was confirmed by surface and bottom gill netting.

The gill nets used were standard commercial nets of No.0, nylon thread, 39mm (knot to knot) supplied by Norsenet, Bergen, Norway. The nets were used as single 25m sections or in gangs containing up to 8 sections, totalling 200m. The 39mm net caught fish between 27 and 41.5cm in length (total) and 138 to 584g in weight (wet). The net sampled the entire range of mature fish, unless it rejected fish over 41.5cm. Throughout the study only four other species were caught in the gill nets: roach Rutilus rutilus (2 specimens at the Ross Islands), perch Perca fluviatilis (10 specimens from Camus an Losgainn to the field station), salmon Salmo salar (3 specimens from Camus an Losgainn to the Ross Islands), sea trout Salmo trutta (22 specimens from the field station to the Ross Islands).

Species present in the study area but not caught in gill nets include: the minnow Phoxinus phoxinus, the sea lamprey Petromyzon marinus, the eel Anguilla anguilla, the three spined stickleback Gasterosteus aculeatus, the ten spined stickleback Pungitius pungitius and the ruffe Gymnocephalus cernua. The following species occur in Loch Lomond but are not known in the survey area: the pike Esox esox, the flounder Platichthys flesus, the river lamprey Lampetra fluviatilis, and the brook lamprey Lampetra planeri.

Results

On 12 November at 13.00 hours, powan Coregonus lavaretus were recorded on a flat zone to the west of the outer Ross Isle (Fig. 18). The fish were at depths of 16 to 18 metres, and close to the edge of a steep slope which dropped to 60 metres. Gill netting revealed that powan were present, during the day and night, in a sex ratio which was close to unity. The area of deep water surrounding the islands was searched at all depths, but no traces of fish were found. The important feature of this recording is the total absence of fish traces in the surface and mid-water layer, which proved to be characteristic of the daytime distribution during the winter months.

To the north of the outer Ross Island is an extensive series of ridges which appear to run in an east - west line. Recordings made over the area during November indicate that powan were present on the bottom during the day and night. Figure 19 records the distribution of the fish before sunset; no fish traces were present in the surface or mid-water layer.

During November, recordings made overnight indicate that the population is divided with fish on the bottom and some in the surface zone. Figure 20 records the presence of powan in the surface zone over deep water, near the Ross Islands.

Recordings made during the night in December close to the outer Ross Island indicate two zones of fish (Fig. 21); one group is on or near the bottom in the 16 to 20 metre zone., and the other is in the surface layer between 0 and 8 metres. Generally, during the winter, powan are distributed evenly between 0 and 22 metres during the night and do not appear to shoal at this time. Surface gill nets caught powan in such small numbers that no meaningful observations could be made on sex ratio.

The area between the outer and inner Ross Islands is known to be a spawning ground for powan (Fuller et al, 1976) but the extent and nature of the spawning substrate is unknown. The bottom characteristics between

the two islands were examined by diving, and the main features are illustrated (Fig. 22). The littoral zone region around the inner Ross Isle is characterised by the presence of the quillwort Isoetes which extends to 3 metres. This plant occured on a fine gravel - sandy substrate; yet observations made immediately after the spawning period (early February) revealed no eggs on either the plants or amongst the gravel. The rocky substrate between the islands did not appear suitable for Isoetes, although there was vegetation (unidentified) to a depth of 10 metres. During the spawning period the greatest number of powan were caught in gill nets, over the rocky ridge, between the islands. The bottom here is mainly exposed rock with a wide range of gravel and loose rocks. There was a gradual slope from 0.2 to 10 metres over the length of the ridge, which is surrounded in every direction by a silty slope. The shallow zone around the outer Ross Island is mainly composed of large rocks and therefore is unsuitable spawning substrate.

It was difficult to find suitable conditions for echosounding during the spawning period, but on 21 December 80 recordings were made over the ridge between the Ross Islands at 22.30 hours. Fish traces were recorded in the surface layers (Fig. 23) but were not positively identified as powan; they resembled traces of powan which had been confirmed by surface gill netting at other times of year. An unusual feature of this recording is the trace above the second echo which had not occurred on previous surveys. If the distribution of fish is so dense that they exceed the resolving power of the echosounder, they can show up as a solid trace although such recordings usually have fish traces at the borders. Dense shoals of fish close to the bottom are known to give rise to secondary echoes (Haram, 1965). During the same period in 1977 and 1978, gill nets set between the islands caught powan in very large numbers. As the recording was made during the known spawning period of the powan it invites the speculation that the males are densely packed and unusually

close to the bottom in some form of spawning distribution as predicted by Fuller et al (1976).

During February 80, the movements of the fish were recorded in Camus an Losgainn. Figure 24 is a record from a series made between 15.00 hours and 09.00 hours. During the night the fish were more or less evenly distributed between the surface and 20 metres; but in the shallow inshore areas with depths less than 15 metres the fish were in the surface 6 metres. The recordings were made during the period of full moon (phase 0.98) on a very cold and clear night with no wind. The number of fish within the surface zone increased progressively from a few traces between civil and nautical twilight to a steady stream of traces after astronomical twilight which continued until dawn. The distribution of fish throughout the recording period is shown in figure 25. Whenever the vertical migrations of the powan were monitored, they always followed the same pattern; fish only appeared in shallow water and in the surface layers during the night and between the periods of nautical twilight.

From January to April, powan were located during the day and night on banks near islands, and on offshore ridges at the southern end of Sallochy Bay and to the north of the entrance to Camus an Losgainn. Figure 26 was recorded during the afternoon (3 April 80) on an offshore ridge. Powan were situated on the bottom between 20 and 24 metres depth. Gill nets set on the ridges during the day and raised after 30 minutes invariably caught powan. No other species were recovered from the nets. No change in this distribution occurred until after the solar altitude reached -6° and was approaching -12°; the fish dispersed and migrated towards the surface. Before the onset of astronomical twilight at 21.12 hours the fish had moved into the surface 10 metres, and during the night were distributed throughout the top 20 metres (Fig. 27). Figure 28 represents the distribution of the fish throughout the dusk period, and relates the migration to solar altitude.

During the months of May and August powan were no longer to be found on ridges or in the region of offshore islands during the hours of daylight; fish traces were only recorded in mid-water layers during the day. Echosounding surveys were not carried out during June and July. Figure 29 was recorded over deep water (60m) at 12.00 GMT on a clear day with minimal surface disturbance. This recording was typical of others made during May and August. The traces were in bands at different levels within the 30 to 40 metres zone but were not positively identified as powan. It seems unlikely that the bands would be caused by plankton or the thermocline which normally only occurred in the top 20 metres; the thermocline in Loch Lomond does not form until June or July. The bands were not found during the night when powan are known to disperse and move to the surface layers. The impression that powan remain in deep water during the warmer summer months might be misleading and due to restricted sampling in the survey area. In the southern basin of Loch Lomond powan occurred in shallow water throughout the day for most of the summer months (Scott, per com.).

During the period after nautical twilight (20.50 hours GMT) on 28 August 80, powan were recorded at depths between 14 to 18 metres over deep water (40m). The recording began at 21.40 hours and continued for thirty minutes (Fig. 30). During this period the fish moved towards the surface layer at approximately 0.3 metres per minute (Fig. 31). Throughout the winter when the fish were feeding on the bottom they were never recorded in shoal formation; the shoal formation indicated in this recording is perhaps related to the pelagic behaviour of the species at this time.

FIGURE 18 12 November 1980, 13.00 hours

Echogram showing powan traces near the bottom on a flat zone to the west of the outer Ross Isle; a typical winter distribution during the daylight hours. No fish were recorded in the surface or midwater layers. A metal object was lowered to establish that the traces were fish.

Scale 1, 0 - 22 metres, white line in operation.

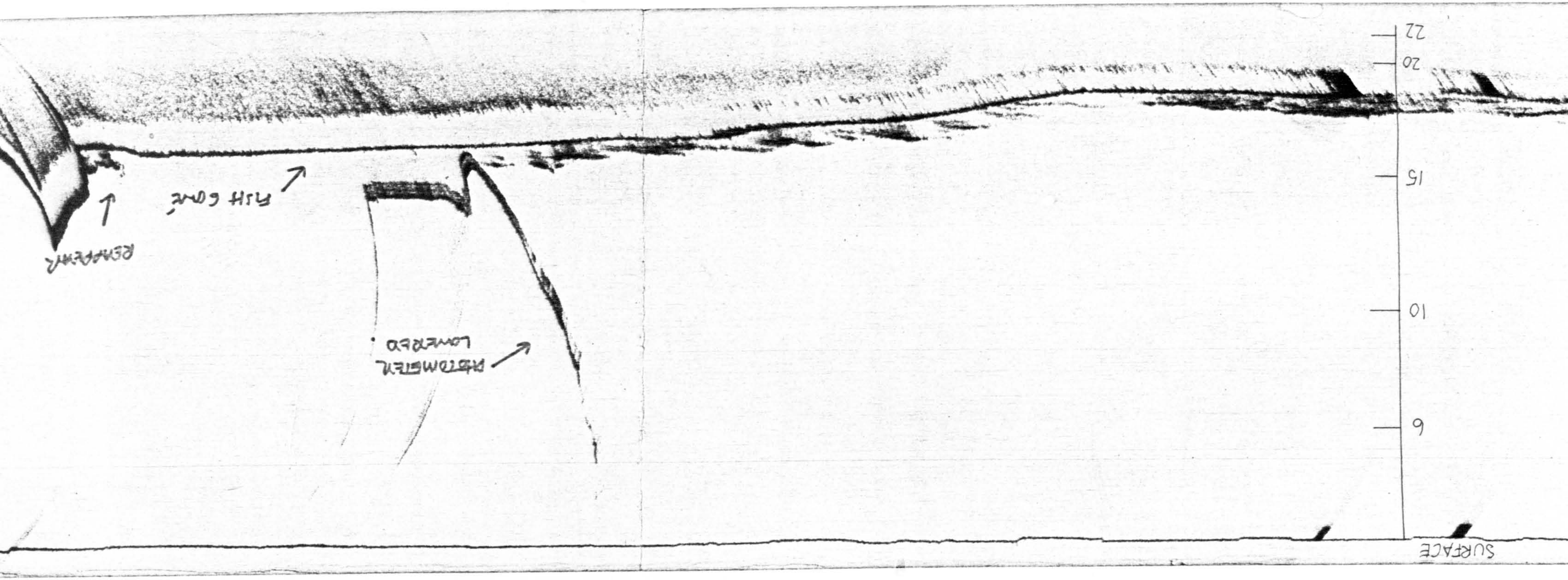

22
20
15
10
6
SURFACE
BOTTOM SILT
LOWER BED
FISH BONE
REMARK

FIGURE 19 28 November 1980, 15.30 hours

Echogram showing powan traces near the bottom in an area directly north of the outer Ross Isle. The recording was made about 45 minutes from sunset (16.14 hours). No fish were recorded in the mid water or surface layers.

Scale 1, 0 - 22 metres, white line in operation.

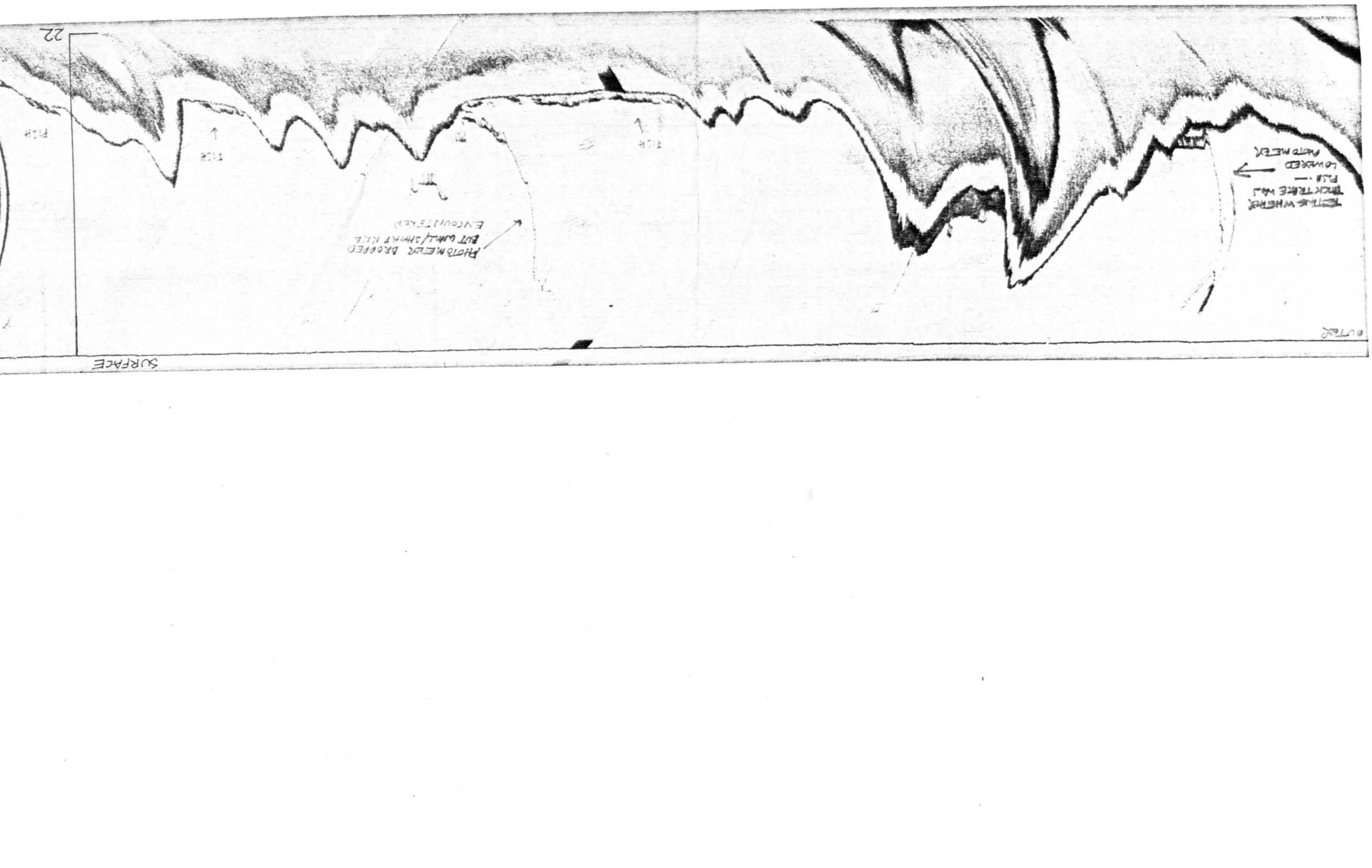
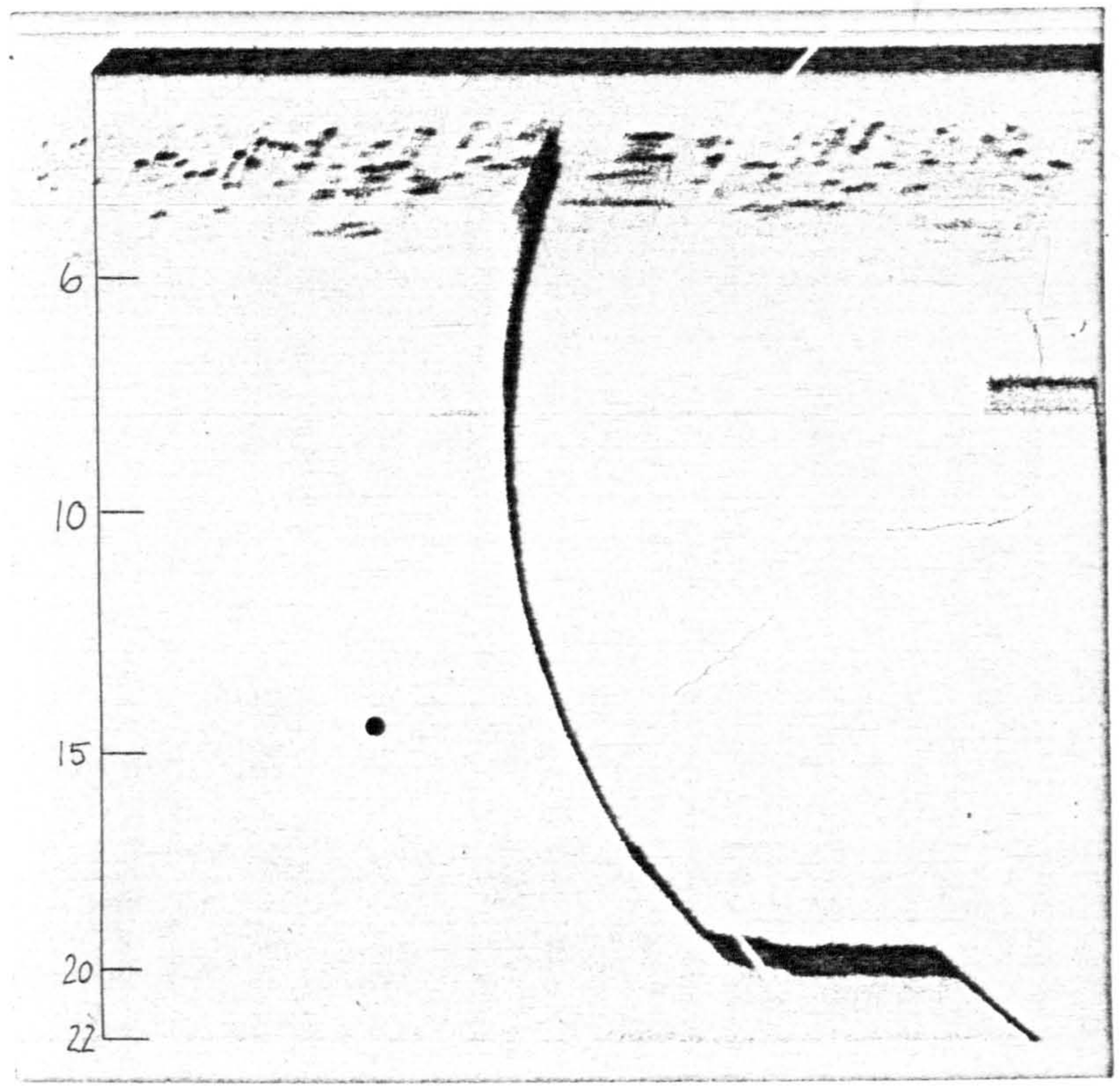

22
SURFACE
PLSH
PLSH
PLSH
PHOTOMETER BROKEN
BUT UMBILICAL/SHORT RISE
ENCOUNTERED
TESTING WHETHER
THICK TREE WILL
PLSH —
LOWERED
METER

FIGURE 20 November 1980,

Echogram showing the distribution of powan

near the surface during darkness.

Scale 1, 0 - 22 metres


6
10
15
20
22

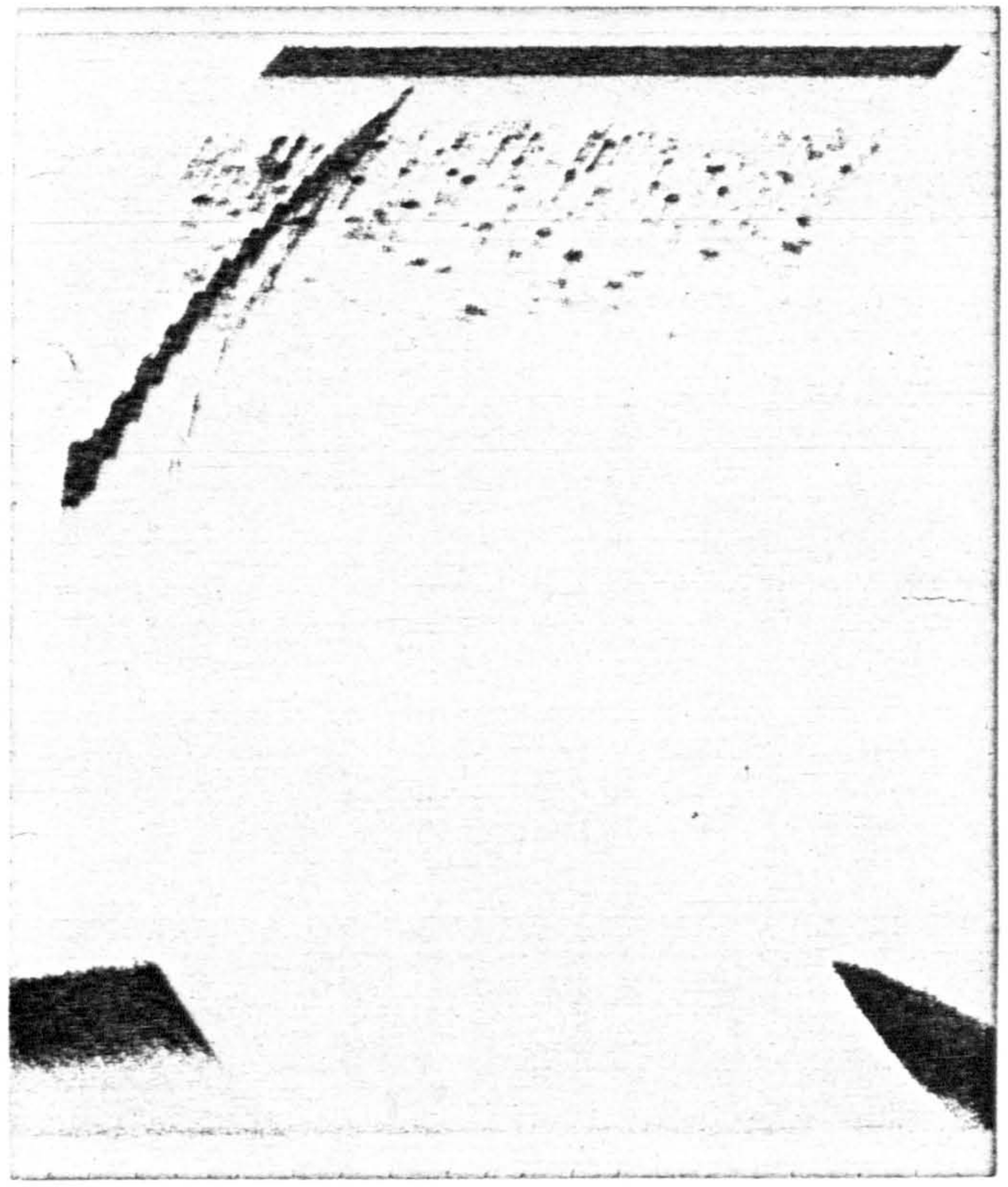


FIGURE 21 19 December 1979, 20.00 hours

Powan at the surface and on the bottom, to the west of the outer Ross Isle. Astronomical twilight began at 17.57 hours. The fish near the bottom have a different appearance from the day time trace; and the fish in the surface layer are characteristic of the night time distribution.

Scale 1, 0 - 22metres

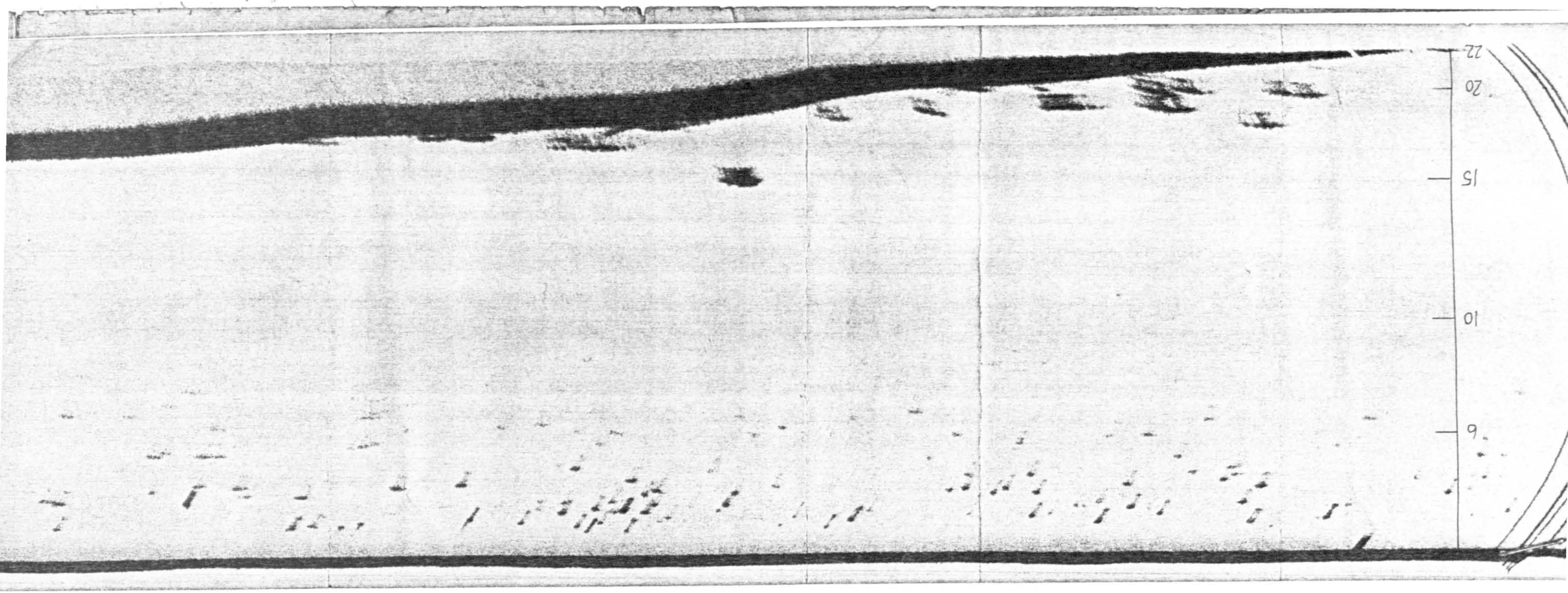
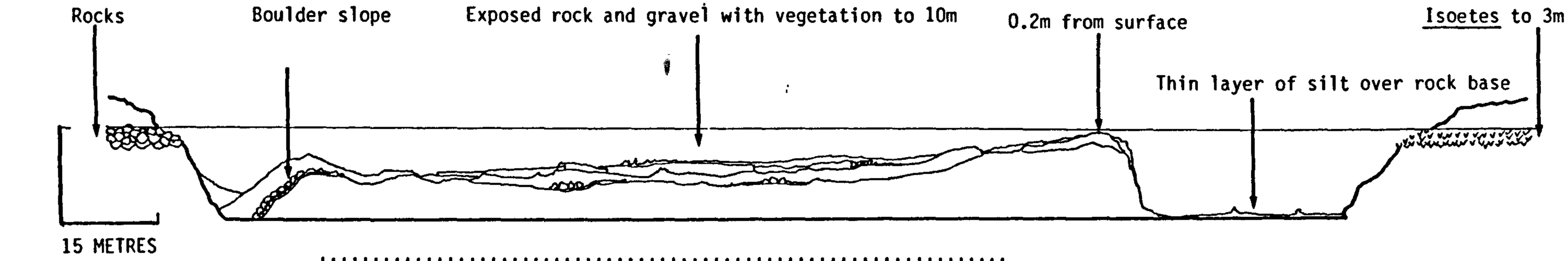

22
20
15
10
9

FIGURE 22 The main characteristics of the rocky ridge between the two Ross Islands. The maximum depth is 15 metres, which occurs at the outer island. The area is surrounded by steep silt slopes. Gill nets set between the two islands during the spawning period 1977,1978 caught fish mainly over the rocky ridge.



BOTTOM CHARACTERISTICS BETWEEN THE ROSS ISLANDS

FIGURE 23 21 December 1980, 22.30 hours

Recording made over the Ross Island ridge at night. Fish traces occur in the mid waterlayer within 6 metres of the surface. An unusual feature of this recording which had not occured on previous surveys, is the presence of a trace above the second echo (⟶).There are also fish traces at the border of the black 'bottom' trace (⟶). There is a possibility that the trace above the second echo may indicate a dense aggregation of fish close to the bottom.

Scale 1, 0 - 22 metres

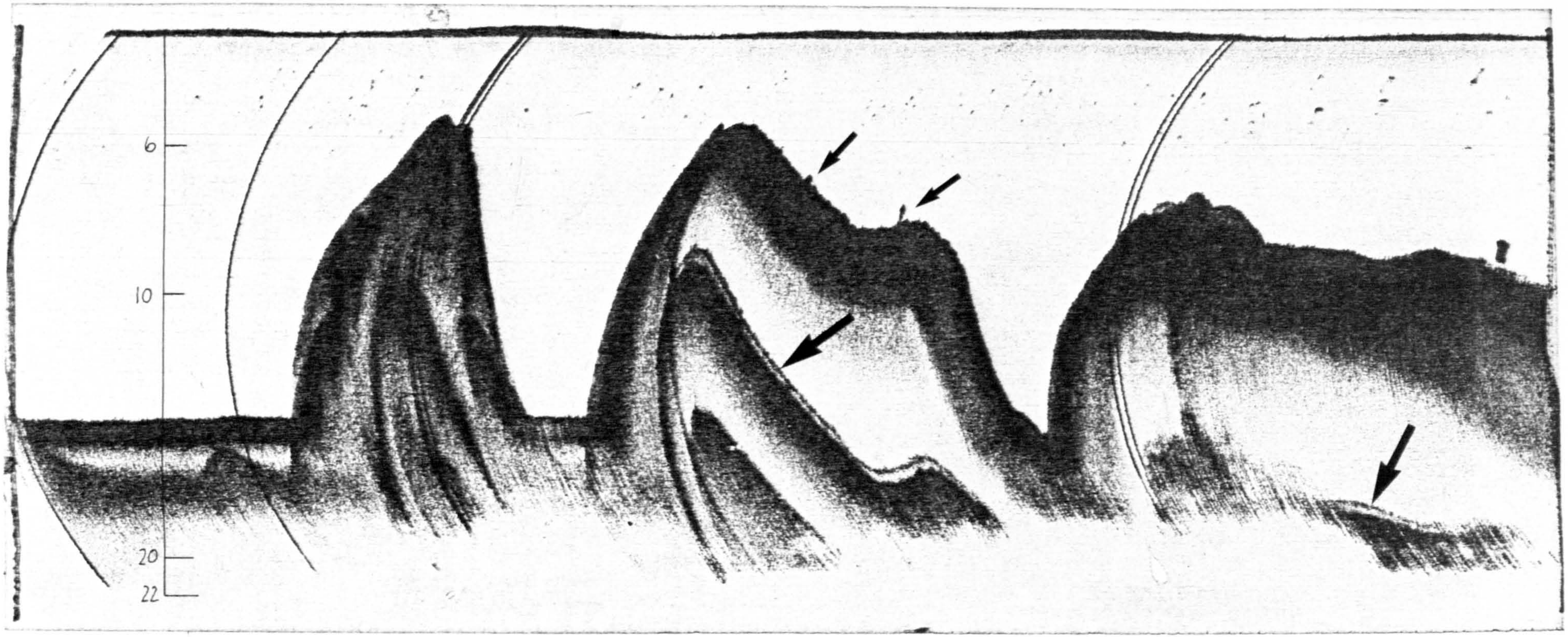

6
10
20
22

FIGURE 24 Late January 1980.

A representative recording of powan in Camus an Losgainn during the night. Fish were more or less evenly distributed between the surface and 20 metres; however, in shallow inshore areas with depths less than 15 metres the fish were concentrated near the surface.

Scale 1, 0 - 22 metres, white line in operation.

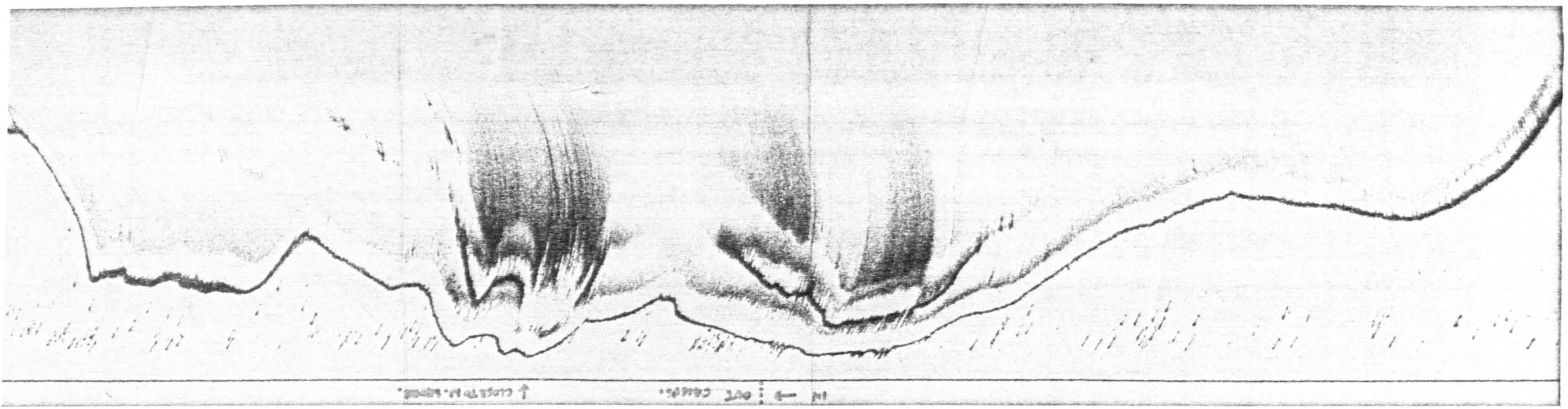


FIGURE 25 Late January 1980, 14.00 - 10.00 hours.

Movements of powan throughout the above period relative to solar altitude. Fish were generally distributed throughout the upper 20 metres in the vicinity of Camusan Losgainn. However, in shallow areas less than 15 metres,the fish were concentrated in the surface layer around the 3 - 6 metre zone. There were,on occasions throughout the night,concentrations of fish at the bottom.

Powan recorded within this area :

Over shallow water and during the day powan concentrated here:

SOLAR ALTITUDE

0° -6° -12° -18° -18° -12° -6° 0°

DEPTH (METRES)

5
10
15
20
25

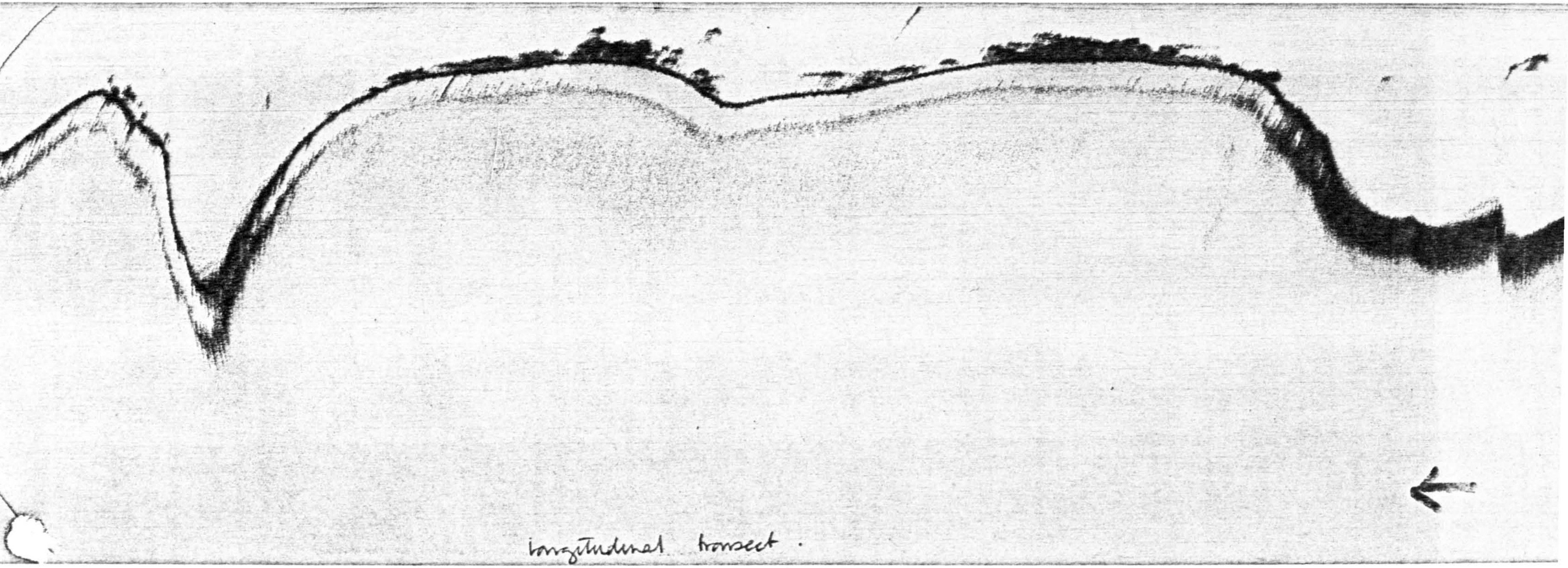
14 16 18 20 22 24 2 4 6 8 10

TIME (HOURS)

FIGURE 26 3 April 1980, 17.30 hours (GMT)

Typical day time distribution of powan during the winter period. The recording shows fish at the bottom, on the ridge outside Camus an Losgainn

Scale 2, 20 - 42 metres, white line in operation.



longitudinal transect.

FIGURE 27 3 April 1980, 20.40 hours (GMT).

A recording at the entrance to Camus an Losgainn showing powan in mid-water between 3 and 10 metres. The recording shown lasts for about 15 minutes and ends at 20.55 hours. The onset of astronomical twilight occurs at 21.12 hours. Throughout the night the fish are dispersed throughout the top 20 metres

Scale 1, 0 - 22 metres.

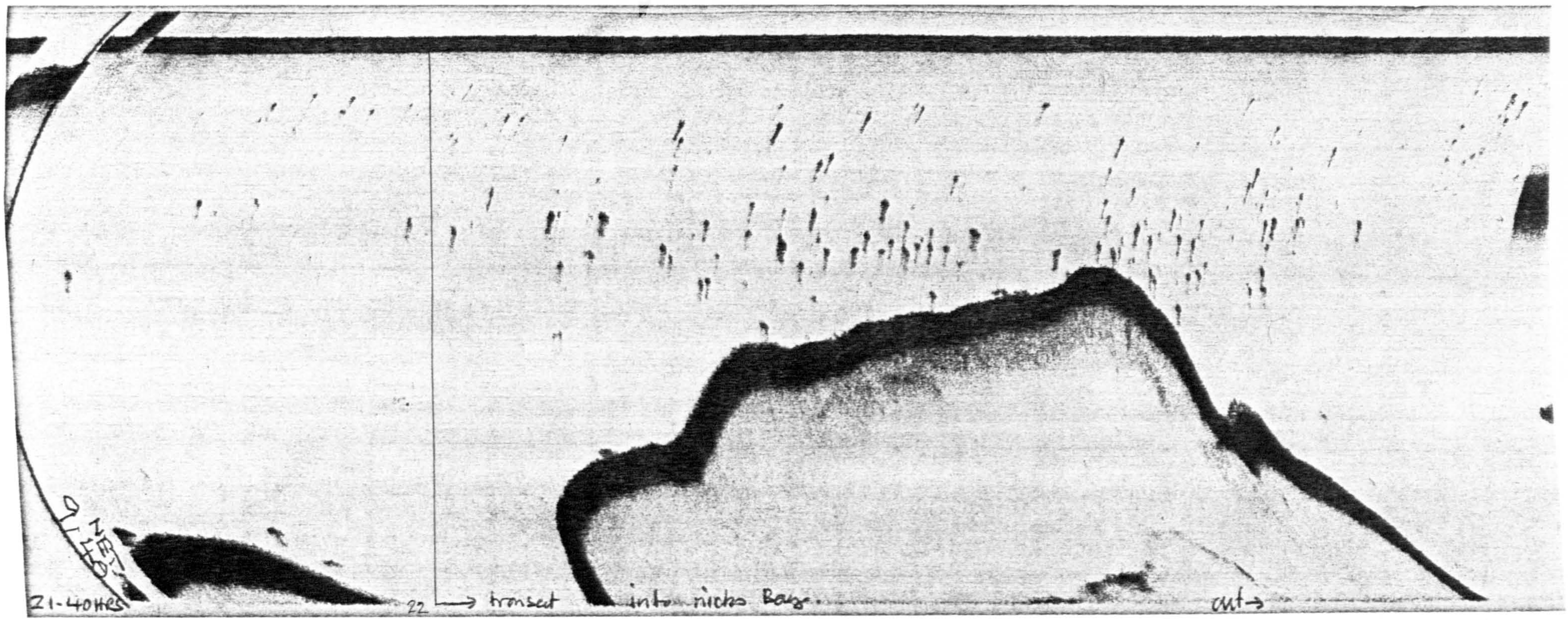

21-40 HRS
22
→ transect into riches Bay.
out→

FIGURE 28 3 April 1980, 16.30 - 21.30 hours

The movements of powan during the above period relative to solar altitude. Throughout the afternoon the fish remained at depth on the offshore ridge to the south of Camus an Losgainn. As twilight advanced the fish made a general movement off the bottom into the surface layers.

Powan were recorded within the area defined by the red line:

Powan mainly concentrated here:

SOLAR ALTITUDE (DEGREES)

0° -6° -12° -18°

DEPTH (METRES)

5
10
15
20
25

16.30 17.30 18.30 19.30 20.30 21.30

TIME (HOURS)

FIGURE 29 18 May 1980, 12.00 hours (GMT).

Fish traces were recorded at various depths within the 30 - 40 metres zone during the daylight hours. This trace is typical of others recorded during the day in May and August. Powan were not positively identified at this depth.

Scale 2, 20 - 42 metres.

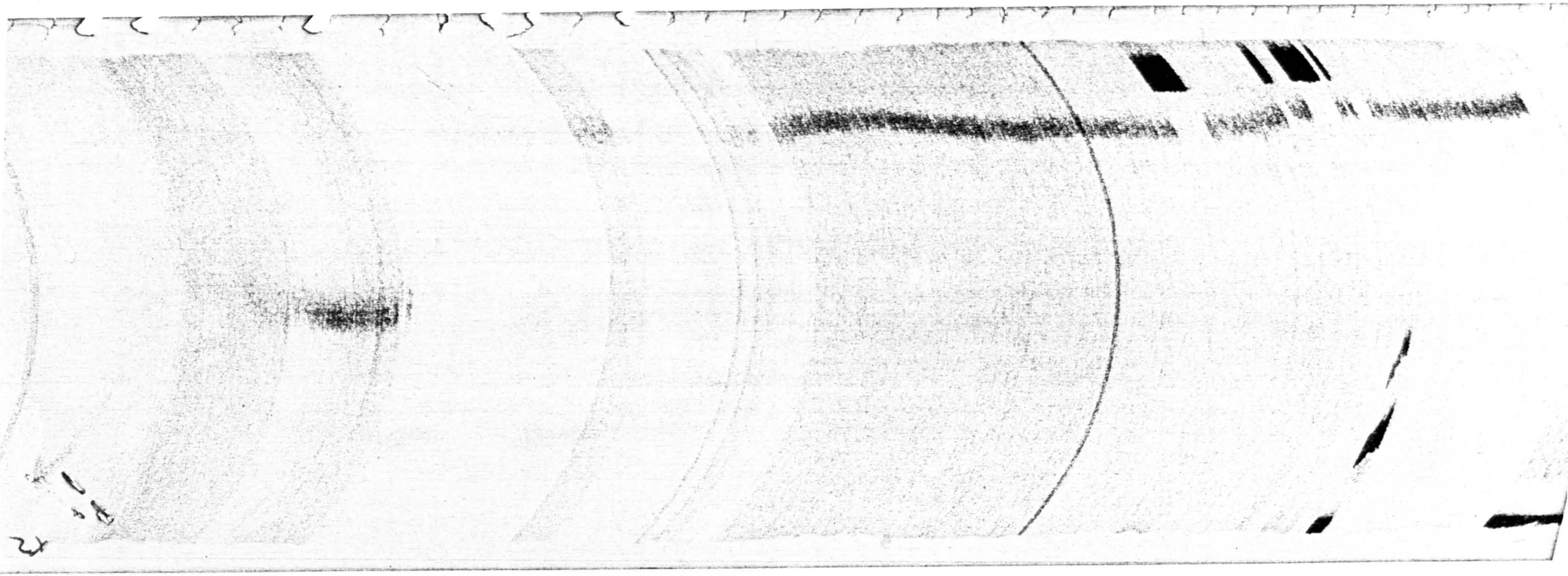


FIGURE 30 28 August 1980, 21.10 - 21.40 hours (GMT)

Powan were monitored during the twilight migration to the surface layer. Nautical twilight occured at 20.50 and astronomical twilight at 21.53 GMT.

Scale 1, 0 - 22 metres

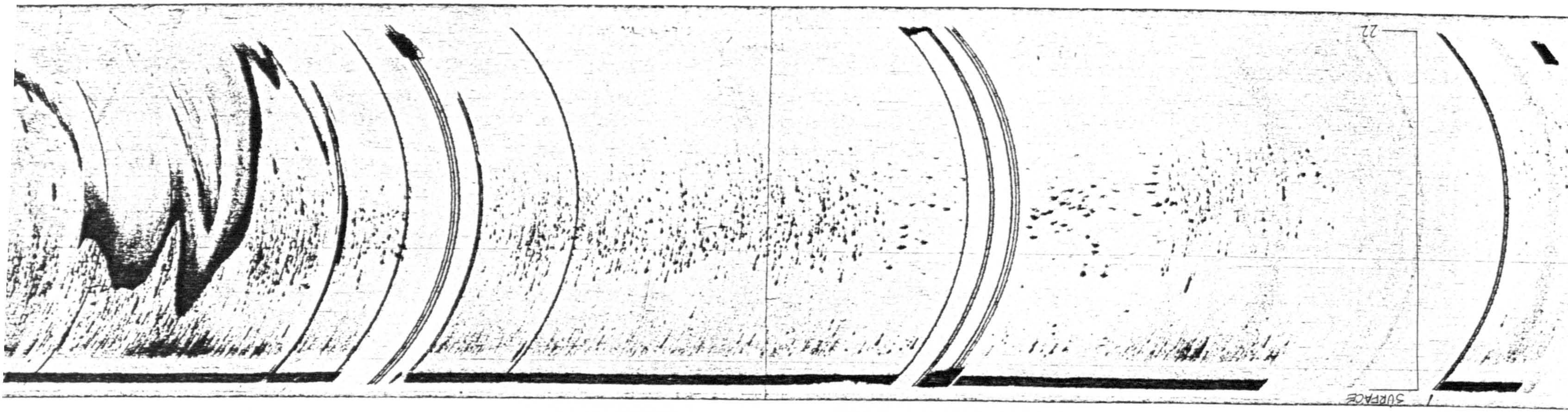
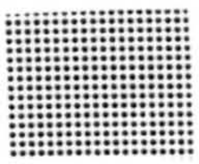

SURFACE
22

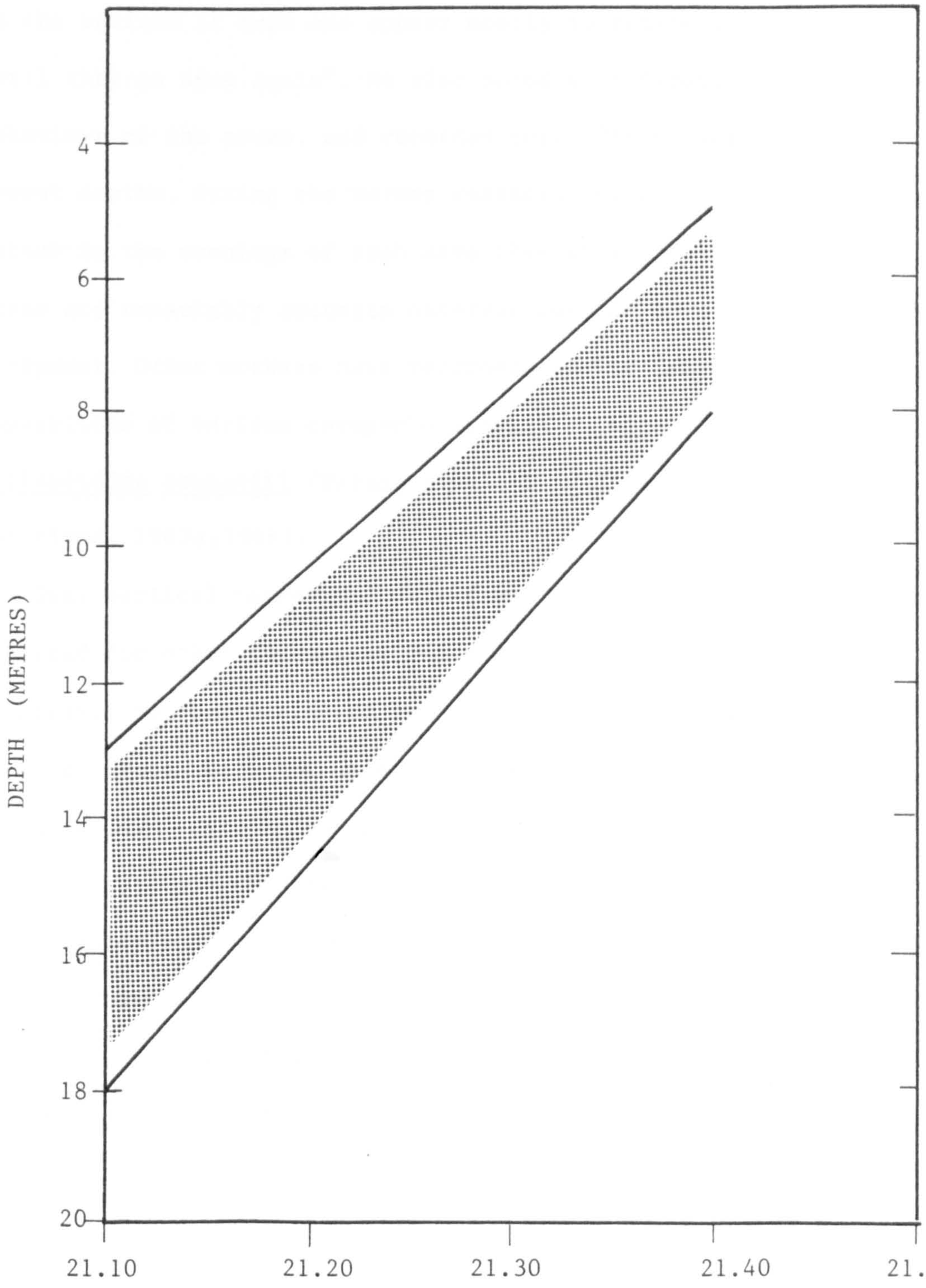
FIGURE 31 28 August 1980, 21.10 - 21.40 hours (GMT).

The evening vertical migration of the powan.

The ascent rate is approximately 0.3m per minute.

Powan recorded within this area: ———

Powan mainly concentrated here: 


4
6
8
10
12
14
16
18
20
DEPTH (METRES)
21.10
21.20
21.30
21.40
21.50
TIME (GMT)

Discussion

The earliest reference to the vertical migration of the Loch Lomond powan is that of Service (1906), who observed that, "the fish move up to the surface at dusk and appear mostly to remain in the surface waters until they go deep again". He also noted a difference in the summer behaviour of the powan, and recorded that, "in summer they remain in the lowest depths, during the warmer weather, but on cold or dull days, or rather in the evenings of such days they will rise higher in the water". These are remarkably accurate observations for someone without echosounding equipment. Other workers have recorded similar behavioural patterns in populations of various coregonid species: C.albula (Dembiński,1971), C.clupeiodes pennantii (Haram, 1968), C.vandesius and C.lavaretus (Maitland, 1967a,1968).

Diel vertical migrations during the dawn and dusk periods, although reported for other coregonids, have not previously been related to solar altitude. The observation of this study, that powan only appear in the surface layers after the onset of nautical twilight (-12°), was supported by shore seine netting experience, where the most successful hauls occured between nautical and astronomical twilight. A similar migration to deeper water occurs at dawn, during the period of nautical twilight and normally before civil twilight.

It may be significant that the form of the migration can differ; during August, when the powan are known to feed predominantly on plankton (Slack et al,1957), they migrate in shoal formation and only disperse after dark. The fish are known to shoal during the day from May to September when their behaviour is mainly pelagic, and obviously related to feeding. In contrast, the vertical migrations in winter seem to involve a general movement of fish off the bottom, with no evidence for shoaling being found. This may be related to their benthic feeding habits; with fish dispersed over large areas of the bottom, in a 'sheet' formation.

The rate of ascent during the dusk migration on 28 August was in the region of 0.3 metres per minute. The powan are known to occupy the 20 - 40 metre zone during the summer. At the above rate it would take 100 minutes to come from 30 metres; which suggests that the migration may have begun between civil and nautical twilight. Similar behaviour occurred during the winter months when they migrated from the 18 - 24 metre zone. The only other comparable information concerns the vertical migration of the North Sea herring Clupea harengus (L.). In autumn, migration covers about 170 metres and in spring 110 metres; highest recorded rates of ascent averaged between 2.3 and 2.9 metres per minute (winter migration) (Falk, 1977).

Although the evidence from this and other studies indicates that whitefish C.lavaretus, do undergo diel vertical migrations during the twilight periods; the Loch Lomond powan occurred in shallow water at other times of day. Powan can be caught in large numbers throughout the day and night by shore seine netting in midsummer (Scott, per com.). O'Connell & Scott (unpub.) have observed surface disturbances during daylight, which probably were powan, but no positive identification was made. There are other accounts of similar behaviour but these are largely anecdotal.

Throughout the study powan were found at the surface during the hours of darkness which is in general agreement with Maitland (1968). Powan were dispersed at night and generally distributed between the surface and 20 metres; concentrations of fish occurred throughout the top 10 metres and often in the surface zone. During an echosounding survey Maitland (1967 a) found that Coregonus vandesius occurred at the surface during the hours of darkness, when the sky was overcast, and during periods of faint moonlight. The fish were identified at the surface by 'dimpling'; although powan were recorded in the top layers of the water during this study, none were observed to break surface. It seems as if powan visit

the surface at some point during the night, and possibly spend considerable periods of time at the surface or in the shallow littoral zone.

From November until the completion of spawning in January, there appears to be a polarisation in the distribution of powan during the night. Fish occur near the surface or on the bottom in the region of the outer Ross Island. Whether this distribution is found at other sites in the loch or elsewhere in the sampling area remains to be established. More work is required to determine the sex ratio in each layer and if the distribution is maintained throughout the above period.

Swynnerton & Worthington (1940), suggested that the schelly Coregonus stigmaticus = C.lavaretus in Haweswater followed the diel vertical migrations of the plankton, and in doing so came close to the surface at night. The evidence from other coregonine populations has demonstrated that the fish feed predominantly on benthic organisms during the winter and that the feeding response generally is depressed (Slack et al 1957; Haram & Jones,1971).Throughout the period May to September the diet of powan consists mainly of zooplankton, and from October to April it is largely benthic. The fact that powan are known to eat benthic organisms during the winter supports the finding of this study, where the fish are found on the bottom in relatively shallow water during the late autumn, winter and spring. It does not explain (a) why the fish persist with their vertical migrations throughout the winter months and spawning period, if they are not feeding on plankton (b) why the migrations should be so precisely timed (c) why they should spend time in the surface layers during the night if their presence is not associated with feeding behaviour.

Zuromska (1982) in a review of factors influencing egg survival provided criteria for the evaluation of whitefish spawning grounds. He concluded that the best (most favoured) spawning grounds are those on a stone or gravel bottom, under a well oxygenated layer, and without strong waving or rapid currents. The rocky ridge between the Ross Islands fulfills

these conditions and would seem to represent an ideal spawning site. Whitefish eggs quickly lose their stickiness and are liable to translocation in areas of turbulence or wave-washed gravel. Eggs ending up on muddy or silty bottoms suffer high mortality (Zuromska, 1982), suggesting that the extensive gravel zone surrounding the inner Ross Isle is unlikely to represent a major spawning site. The area generally is surrounded by silt slopes.

CHAPTER 2

Echosounding

Summary

Evidence of diel vertical migration was found. Powan migrate to the surface layers and inshore during the evening twilight and return to deeper water at dawn. The migrations appear to be precisely timed and related to low light intensities. Fish appeared in the surface layers during the night and within the periods of nautical twilight. Migrations to the surface and shallow water during the day in summer are known and very probably related to feeding behaviour which is pelagic at this time. The diel vertical twilight migrations were recorded during May and August.

The powan appear at the surface during the night and persist with the diel vertical migrations during the winter months; at a time when they are known not to feed on plankton and when the feeding response generally is depressed.

CHAPTER 3

Environmental Control Experiments

Introduction

There are many studies on the environmental control of reproduction in teleosts (reviewed, Pickford and Atz, 1957; de Vlaming, 1974) and in particular on the effect of photoperiod (recent bibliography, Htun-Hun, 1977). It is clear that the timing of teleost reproductive cycles is influenced by environmental factors. However, the precise nature of these factors and the mechanisms of interaction with the physiology of the animal remain confused. In part this is due to the wide range of species investigated, each having evolved a reproductive strategy for its own unique environment. Moreover, the reproductive physiology of a species will be adapted to the needs of that strategy. It is mainly for these reasons that Scott (1979) points to the incompatibility of data in reviews of the subject.

The interaction between the fish and the environment it inhabits is a subtle one. It is known that for many temperate and artic zone teleosts, the regulation of the reproductive cycle involves a complex interaction of temperature and photoperiod (Peter & Crim, 1979; Crim, 1982). The matter is further complicated as different stages within the cycle such as vitellogenesis, final maturation, ovulation and spawning are under independant control and may require separate cues (de Vlaming, 1983). The precise nature of the cues used by teleosts has remained elusive; photoperiod for example is generally referred to as 'summer or winter' or 'increasing and decreasing'. These vague descriptive terms mask the subtleties of the environment, especially photoperiod.

The importance of the twilight period in the assessment of daylength and entrainment of endogenous circadian rhythms has been demonstrated in a teleost (Kavaliers & Ross, 1980). Recently, it has been shown that photon fluence rates during the twilight period can provide threshold

time signals accurate to 10 minutes or less (Hughes et al, 1984). It may be therefore that misleading data are generated by exposing fish to fixed photoperiods which (a) ignore seasonal variation in responsiveness and (b) exclude the seasonally variable twilight period.

This part of the study examines the effect of photoperiod and temperature on the reproductive cycle of a temperate zone teleost. Unfortunately the powan Coregonus lavaretus was unsuitable but the common sole Solea solea (L.) proved a suitable alternative.

Studies on sole date back to 1890 when Cunningham first noted that spawning occurred once a year during the spring. Subsequently, Shelbourne (1968) showed that sole would spawn naturally in captivity. More recently an effect of photoperiod on the timing of spawning has been demonstrated (Bye & Htun-Han, 1979).

Materials and Methods

The species

The species chosen for this part of the study was the Dover sole (common sole), Solea solea (L.), family Soleidae, a temperate zone fish with an uncomplicated reproductive cycle. The fish is hardy, easy to feed in captivity, and has a spawning period of four to six weeks; it also has the decided advantage of spawning spontaneously in captivity. Ideally, it would have been preferable to use Coregonus lavaretus for the experiments but the species does not travel well, is easily stressed, difficult to feed as an adult, and its spawning behaviour in captivity is unknown.

Water supply

Each tank was supplied with sea water from two sources: coastal sea water pumped from a depth of eight metres which followed the natural temperature cycle, and power station effluent water which is normally 8°C above the temperature of the sea water. By mixing the two flows any required temperature could be reached. Pressure changes in the water supply could create an imbalance in the mixture, resulting in a temperature change of 1 to 2°C, but these were of a random nature. The volume of the fish tanks was large (4 to 25m^3), and flows small (0.6 to 4m^3/hour), therefore the changes in temperature were gradual; occurring over many hours.

Photoperiod

Each photoperiod cell was completely lightproof and under independent control. The natural cycle of photoperiod was controlled by a Sangamo Western Solar Dial time switch, set for a latitude of 56°N. Time clocks were controlled through a dimmer unit which provided a 20 to 30 minute twilight period.

For a latitude of 56°N, the range of daylengths at various negative solar altitudes on 21 September were as follows:-

Solar Altitude	-18°	-12°	-6°	-0°
Daylength (hours)	16.7	15.1	13.6	12.4

The Sangamo Solar Dial switches have the facility to allow adjustment for longitude up to 45 minutes either side of 56°N. With the dimmer unit they allow the simulation of various twilight periods.

Light levels were kept low, between a maximum of 80 lux and a minimum of 20 lux at the water surface (day values). Lighting was provided by incandescent bulbs rated at either 40 or 60 watts in overhead light fittings, which were positioned approximately one metre above the water surface.

Tanks

The experiments on environmental control were carried out in a variety of tanks, the smallest having a volume of 4 m^3 and the largest 25 m^3. The depth ranged between 80 and 100 cm. All tanks were completely enclosed, either as a single unit or within a photoperiod cell.

Food

Depending on availability, the fish were fed on either the lugworm Arenicola marina, the common cockle Cardium edule, or a pelleted diet (White Fish Authority). The diet was supplemented from time to time by organisms brought in through the sea lines. Newly moulted crustaceans, such as the shore crab Carcinus maenas,were a favourite food source for Solea solea. Very occasionally severe weather prevented the collection of food, but this rarely stopped feeding for more than a few days.

Stock collection

Both the established stocks and the new recruits used in this study came from three sources: Irish Sea, North Sea, and the English Channel. Fish were collected from fishing boats and transported north within 12 hours of capture. Shortly after arrival, all the fish were given fresh water baths to remove ecto-parasites (15 to 25 minutes). This treatment was normally very successful and re-infestations were rare.

Stocking Density

In the experiments described the stocking density of each tank was kept low. The smaller tanks (4-8 m^3) were maintained at 0.4 - 0.5 Kg (wet weight) per m^3. The larger tanks (10-24 m^3) were stocked at up to 1.2 Kg per m^3.

Fish were recruited to the spawning stocks if their total length was equal to or greater than 26.5cm. The heaviest females stocked were between 1.4-1.6Kg.

Sex Ratios

In all experimental tanks an attempt was made to have a sex ratio of 1:1. The distinctive swelling of the ovaries during late vitellogenesis provided a means of confirming the number of females in a stock.

Spawning History

With the exception of the fish used for experiments 4 and 5 all other stocks spawned previously at the natural time ie April-May.

Experiment 4 (a-c) The initial change in photoperiod from 18.5 hrs light was applied, simultaneously to all tanks, 140 days from the start of the previous spawning period.

Experiment 5 The phase change in the photoperiod was applied 139 days from the start of the previous spawning period.

Spawning

One month before the fish were scheduled to spawn, flows into the tank were reduced to a trickle overnight. The ova of Solea solea are pelagic and float to the surface (forming rafts) in still water. Occasionally ova which were viable had density problems and remained in suspension or sank to the bottom. Maintaining a small water flow through the tank over-night created sufficient turbulance to keep the ova in motion and ensured that they would be seen.

Results

Experiment 1 : A control for all other experiments (Fig. 32). The fish were maintained on a photoperiod, which simulated natural daylength, and exposed to a natural cycle of temperature. The minimum temperature was 5.5°C, and the maximum temperature was 18°C; for most of the year temperatures ranged between 9 and 14°C. The population used in this experiment had already spawned successfully at the completion of the previous reproductive cycle; no change was made to the photoperiod or the temperature. The spawning period started on 11 April during a period of increasing temperature and photoperiod, and was not significantly different from the previous cycle. The spawning period lasted for six weeks; the majority of ova were produced during the first four weeks.

Experiment 2 : A control to all experiments carried out at a maintained temperature of 13°C (Fig. 33). Temperature throughout the experiment was in the range 11 to 14°C with a mean value of 13°C. The photoperiod simulated natural daylength and was identical to experiment 1. The spawning history, and origin of the population were identical to the fish used in the first experiment. Spawning occurred on 10 March and lasted for six weeks; the majority of ova were produced during the first four weeks. The spawning date represented an advance of about one month over the previous year and in relation to the performance of the fish in experiment 1. The difference in spawning dates suggests a causal relationship with the sea water temperature.

Experiment 3 : The objective was to test if an advance in spawning period could be achieved by photoperiod manipulation. Temperature conditions as for experiment 2 (Fig. 34). After completion of the previous reproductive cycle the population were maintained on a natural cycle of photoperiod. At the beginning of August the population was subjected to a phase shift in the photoperiod; this was achieved by altering the time clock to the equivalent of the autumnal equinox (21 September) on 1 August. The spawning

period began 177 days after the phase shift, representing an advance of 77 days over the population under natural conditions (experiment 1). When compared to experiment 2 the advance was reduced to 45 days. Spawning activity continued for six weeks; ova were produced throughout this period.

The results provide evidence that the time of spawning (at temperatures greater or equal to 8°C), can be influenced by photoperiod manipulation at an early stage in the reproductive cycle.

Experiment 4 : Three populations were maintained on 18.5 hours photoperiod from the previous spawning. Simultaneously, each group began a programme of decreasing photoperiod (manually adjusted). The rate of change for each group was different: 1 hour/week, 1.5 hours/week, and 2 hours/week. Temperatures were maintained at 13°C. One population began spawning after 176 days and the others after 178 days. These results agree with experiment 3 and suggest that the period between cue and spawning is close to 180 days. The fact that all three groups began spawning at the same time suggests that the initial change in photoperiod may have acted as a photostimulatoty cue (Figs. 35 a,b,c).

The fish in each group had a similar spawning history, and the possibility that the populations were not primed to respond to a photostimulatory cue when the experiment began cannot be eliminated. If this occurred, the period between cue and spawning should be shorter.

If the interval between cue and spawning is less than 180 days there is a possibility that a specific daylength, rather than change in daylength, may have acted as a cue. The effect should have been to create a stagger in the date that spawning began, but individual variation within groups may have concealed this. The results of this experiment indicate that spawning can occur within 180 days of a photoperiod cue.

Experiment 5 : In this experiment the population were exposed to a phase shift from a constant 18.5 hours photoperiod; the long day photoperiod had

been maintained since the previous spawning. The daylength was changed from 18.5 to 14 hours, and spawning began 162 days later (Fig.36). After the phase shift the photoperiod followed the natural cycle and temperature was maintained at 13°C (temperature details as for experiment 2). The spawning period lasted for six weeks; ova were produced throughout the period.

The results suggest that the period between the photoperiod cue and spawning can be as short as 162 days, which falls within the period of 150 to 180 days between minimum and maximum gonadosomatic index (Fig.40). The fish in experiment 2 spawned under constant temperature on 10 March which suggests that the photostimulatory cue in the natural environment occurred during late September.

Experiment 6 : The effect of constant photoperiod was investigated. Fish were recruited from the wild, immediately after their spawning period, and placed on a continuous photoperiod of 24 hours light (Fig.37). Temperature was maintained at 13°C throughout the experiment; variation occurred in the range 12 to 18°C. The population began spawning on 7 March; ova production was more or less even for the first six weeks, then occurred sporadically for several months. The fish began spawning close to their 'normal' time but the duration of the spawning period was greater than for populations maintained on a natural photoperiod.

Experiment 7 : A similar experiment to 6 except that the fish were maintained on a continuous 'summer' photoperiod of 18.5 hours from the time of capture (Fig. 38). The population began spawning on 9 March but as in experiment 6 the spawning period extended for several months.

Experiment 8 : The object of this experiment was to establish if a delay in spawning could be achieved by a late phase shift. Temperature conditions were as for experiment 6. The phase shift was made six months before the target spawning time of June - July (Fig.39). The fish were maintained from the previous spawning on a 'summer' photoperiod of 18.5 hours. At

the phase shift the population were exposed to a daylength which was the equivalent of the autumnal equinox.

The results indicate that a late phase shift in the photoperiod had no effect on the success of spawning; sole spawning behaviour continued during a winter photoperiod. Sole also spawn successfully during 24 hours light. A change in photoperiod during the period of exogenous vitellogenesi: has no effect on the progress of the reproductive cycle.

The fish began spawning on 9 March, and continued for several months. The results indicate that sole will continue with their reproductive cycle in the absence of a natural cycle of photoperiod and as in experiments 6 and 7 the spawning period is extended. It is concluded therefore that sole are refractory to photostimulatory photoperiods during the periods of exogenous vitellogenesis, final maturation, ovulation and spawning.

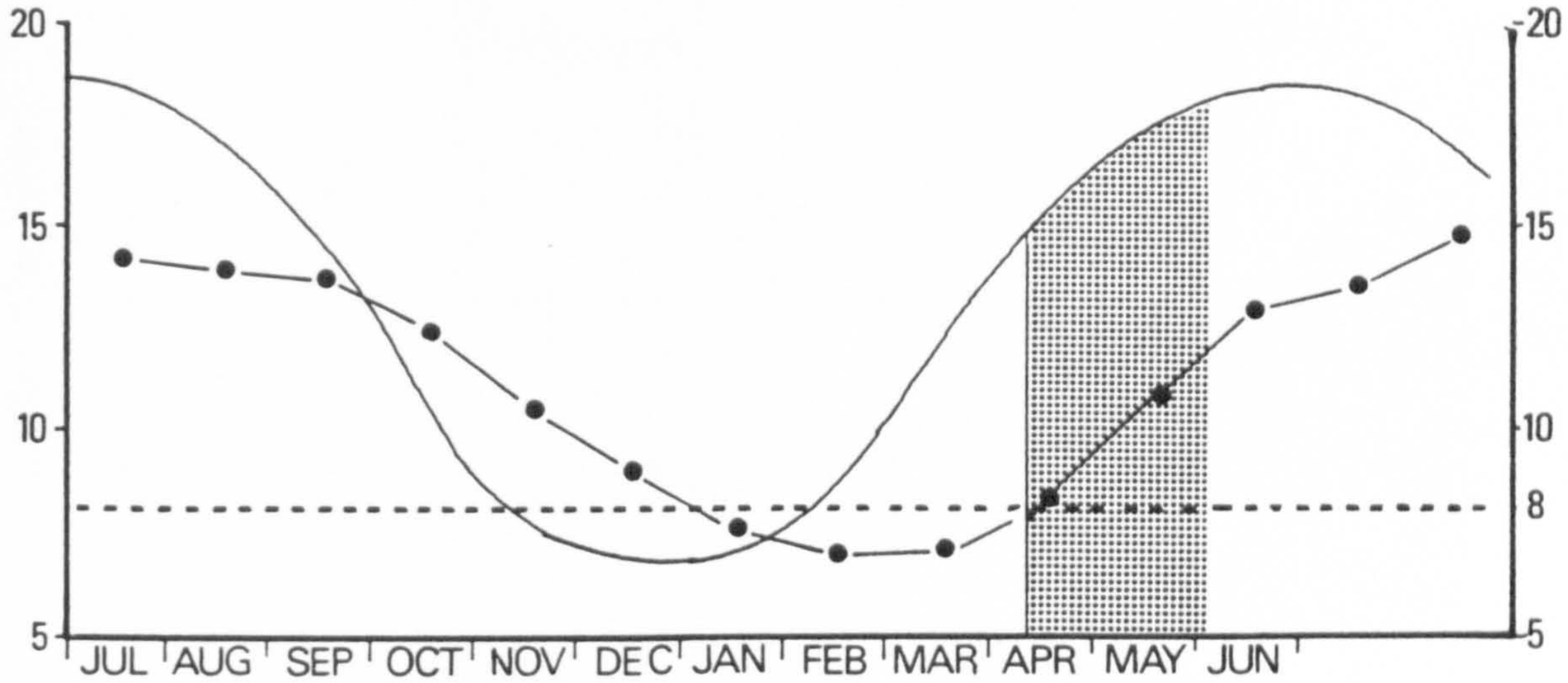
Reproductive cycle : The gonadosomatic index of female sole is shown in (Fig.40). The value remains close to 1.5% from April to September (Deneil, 1981). Between September and October the value increases significantly and continues to increase until February.

The condition factor (including gonads) of female sole was recorded over 10 years (1963 - 1973) and is shown in figure 41 (de Veen, 1976). The value increases slightly between July and August, but a large rise occurs between August and September.

FIGURE 32 Experiment 1 : The fish were exposed to a photoperiod which simulated natural daylength and a natural cycle of temperature: mean monthly temperature (black dots), spawning period (stippled).

FIGURE 33 Experiment 2 : The fish were exposed to a photoperiod which simulated natural daylength and the temperature was maintained close to 13°C (range 11 to 15°C): mean monthly temperature (black dots), spawning period (stippled).

FIGURE 34 Experiment 3 : The fish were exposed to a photoperiod which simulated natural daylength. The phase was changed at the beginning of August. Temperature was maintained close to 13°C (range 11 to 15°C): spawning period (stippled).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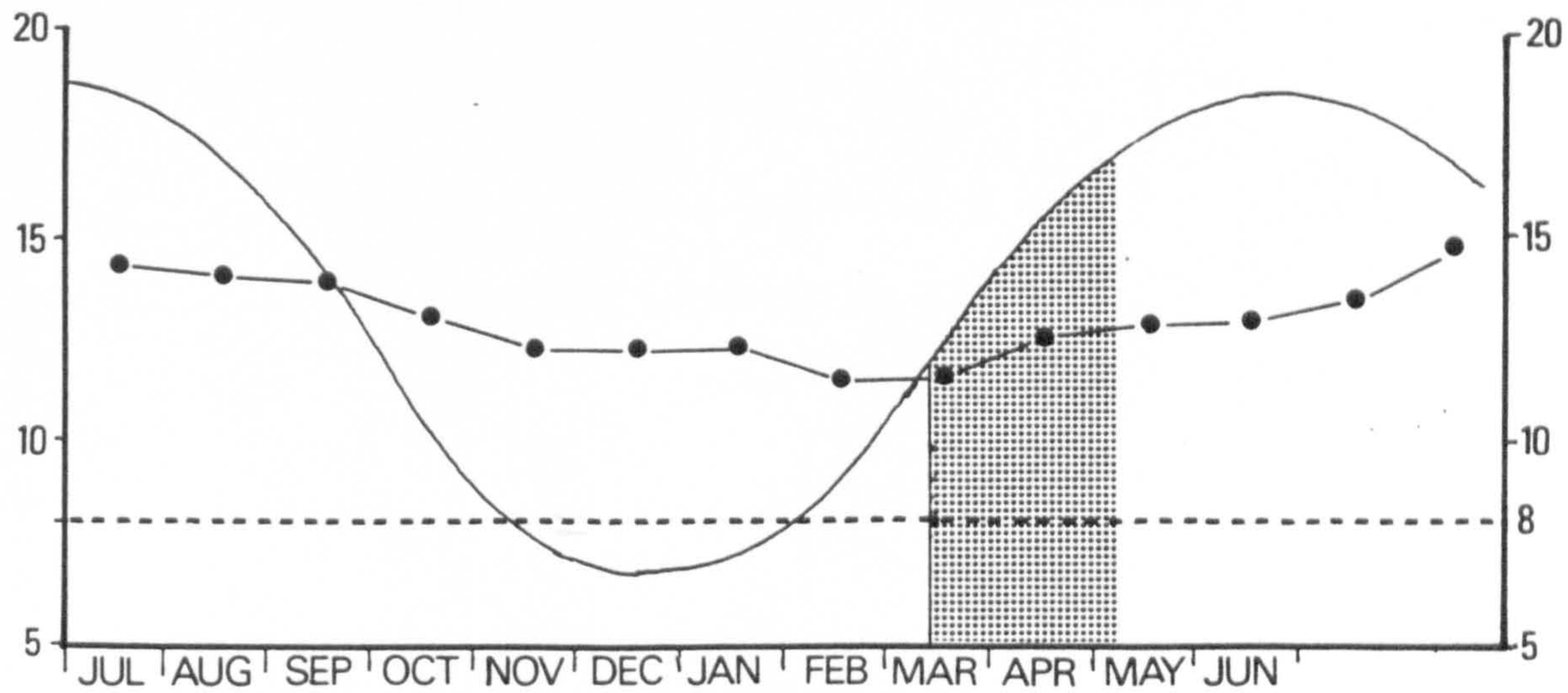
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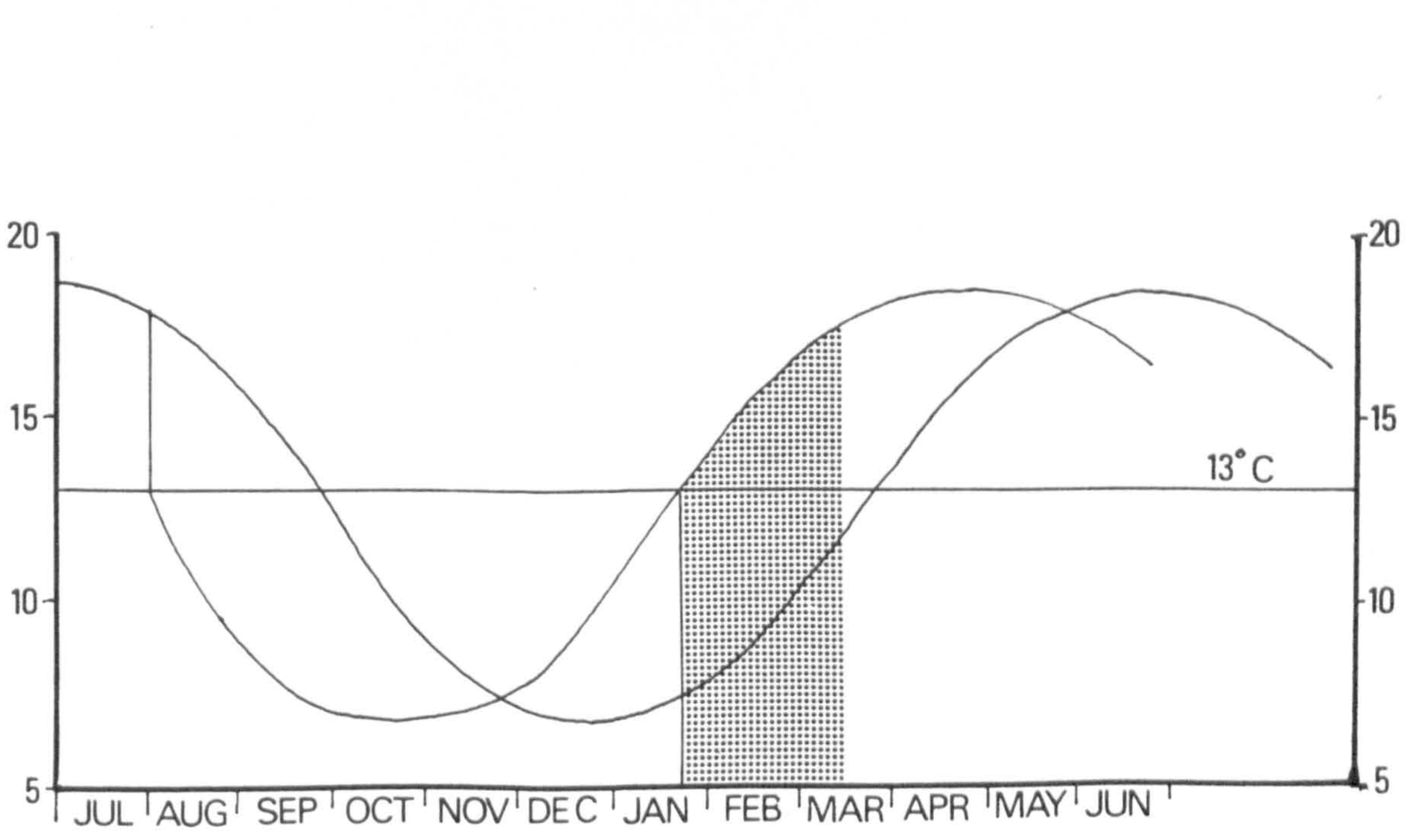
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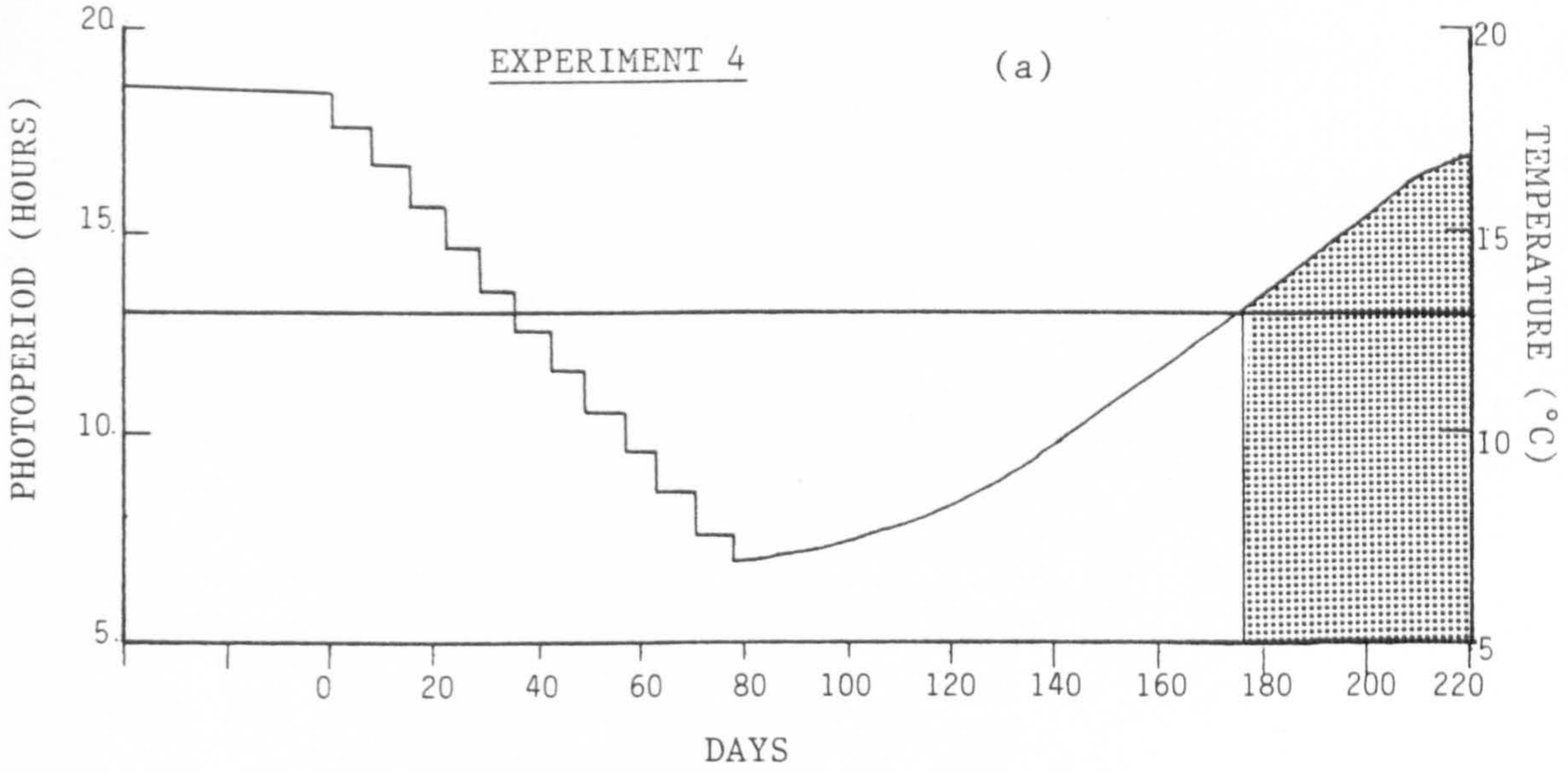
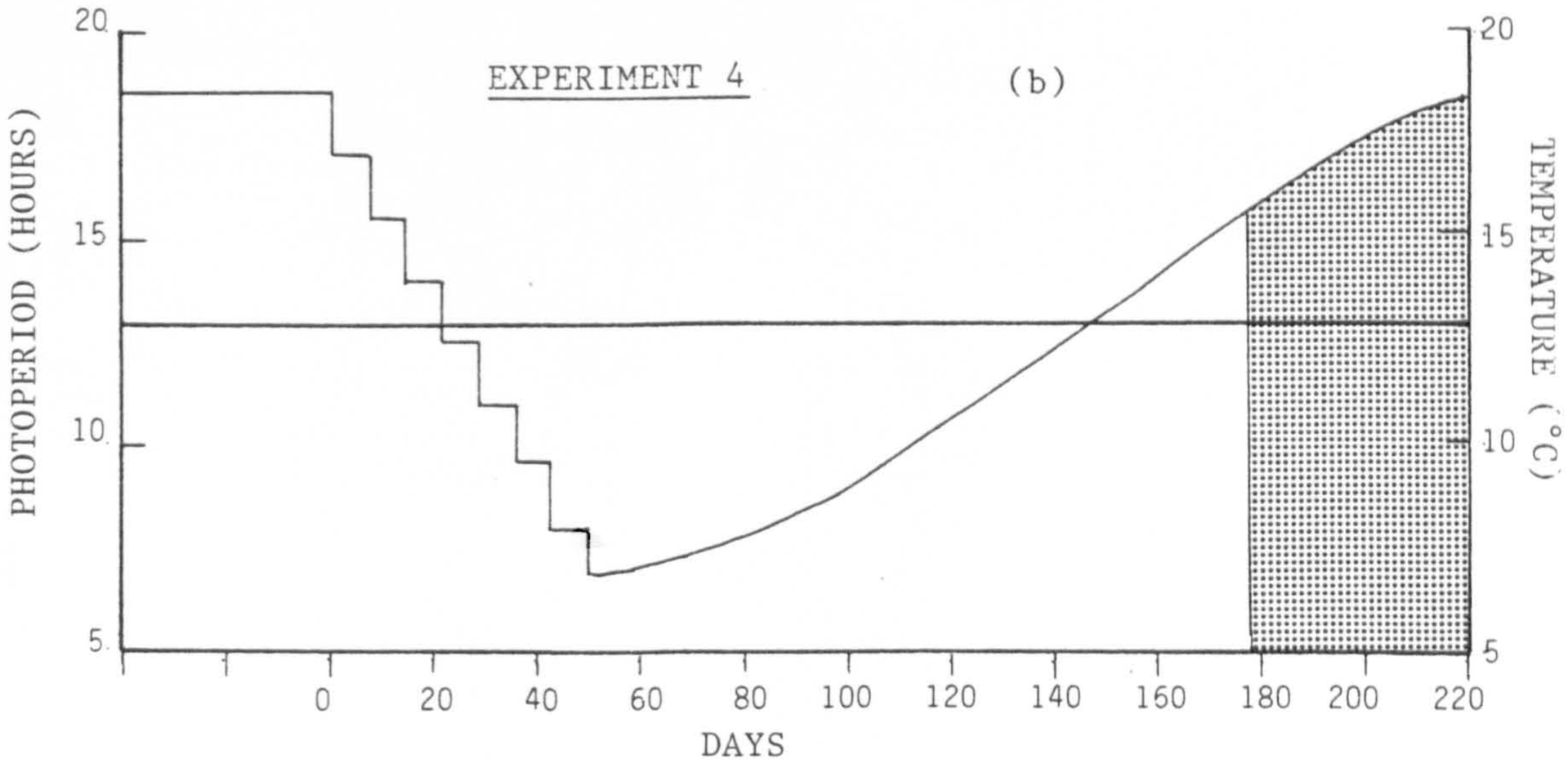
FIGURE 35 Experiment 4 : The photoperiod was reduced at different rates from 18.5 hours to 7 hours. The photoperiod was then changed to simulate natural daylength. Temperature was maintained close to 13°C (range 11 to 15°C): spawning period (stippled).

a = 1 hour/week

b = 1.5 hours/week

c = 2 hours/week.


EXPERIMENT 4
(a)
PHOTOPERIOD (HOURS)
TEMPERATURE (°C)
20
15
10
5
0
20
40
60
80
100
120
140
160
180
200
220
DAYS


EXPERIMENT 4
(b)
PHOTOPERIOD (HOURS)
TEMPERATURE (°C)
20
15
10
5
0
20
40
60
80
100
120
140
160
180
200
220
DAYS

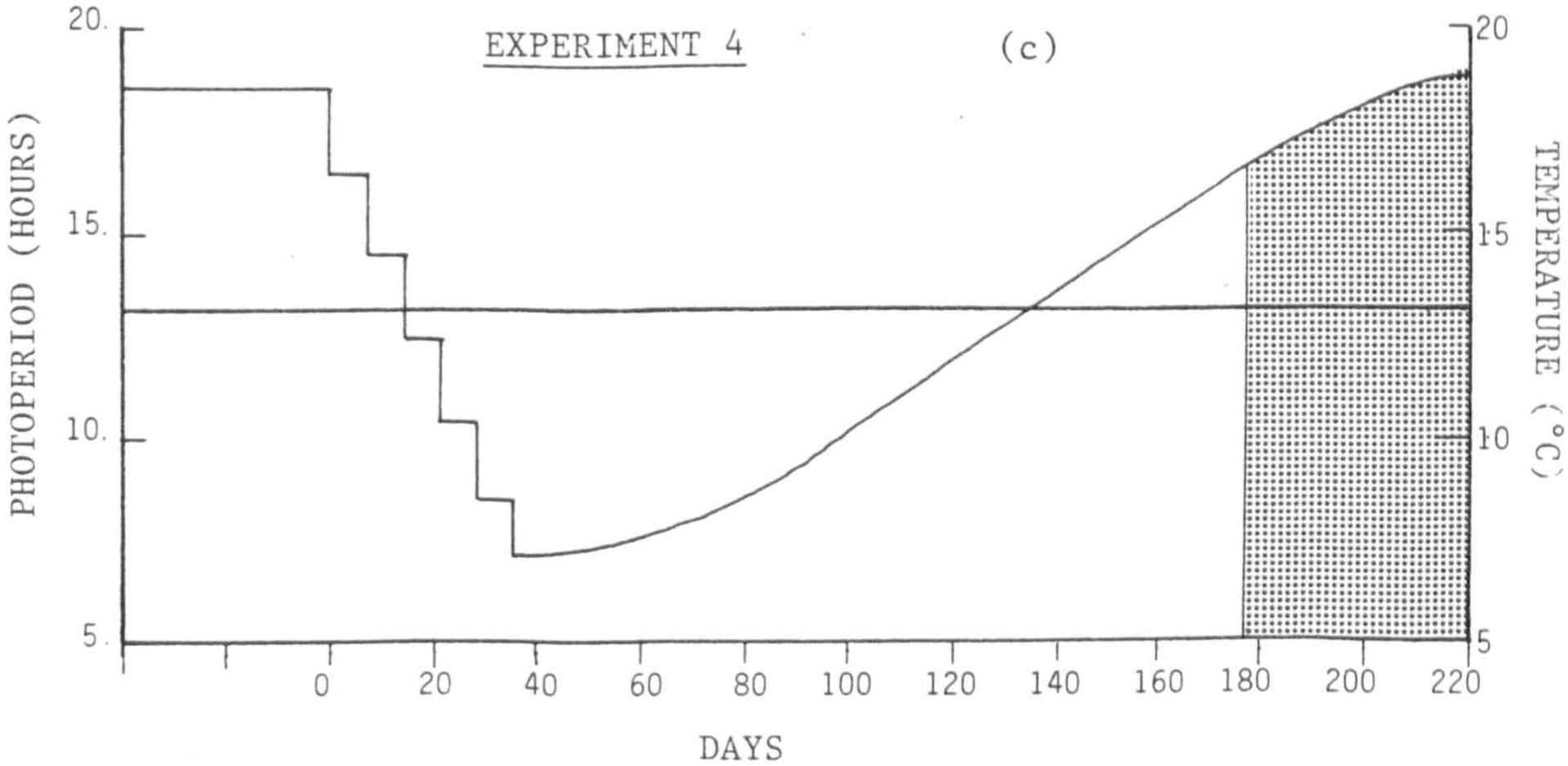
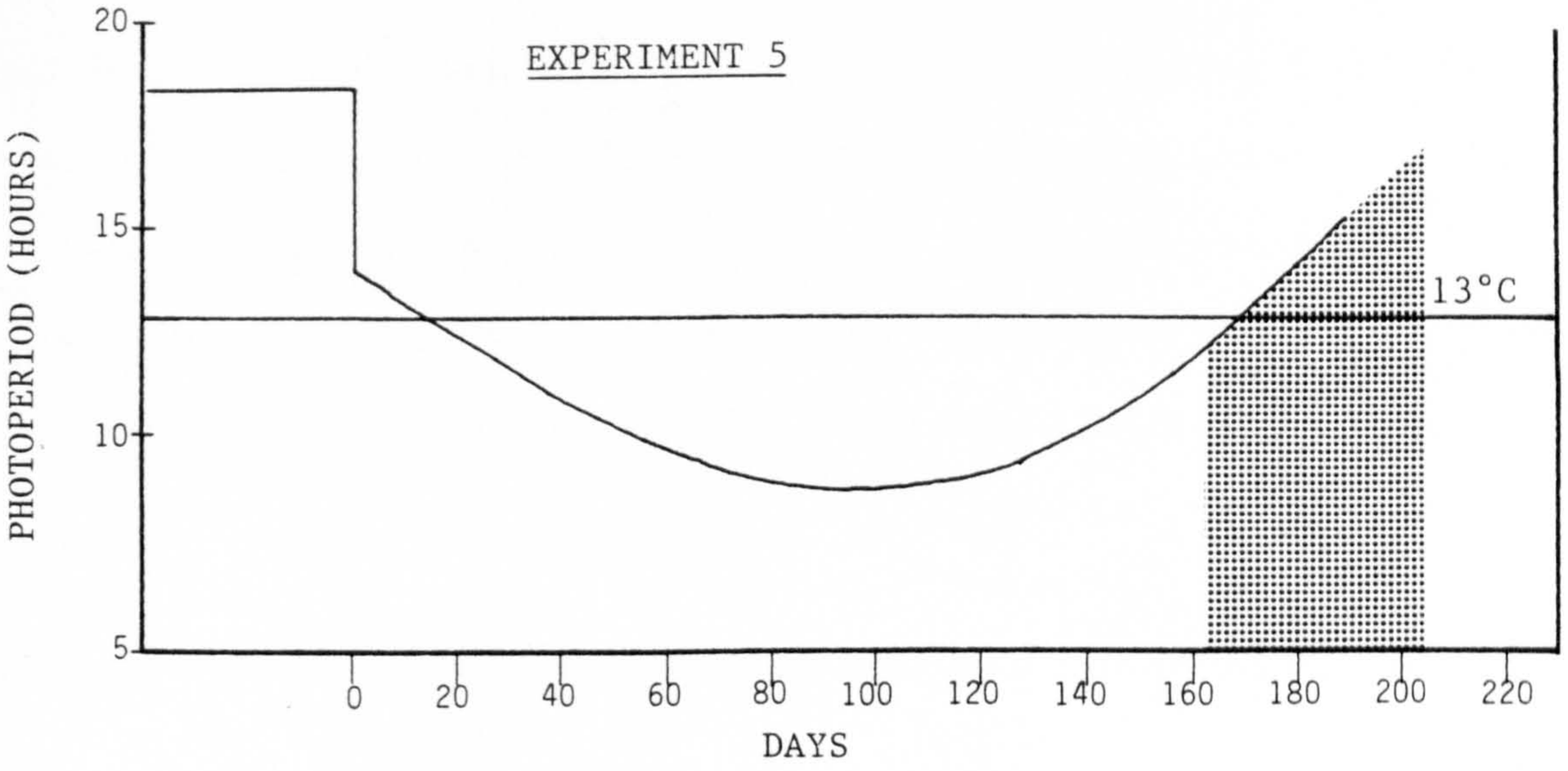
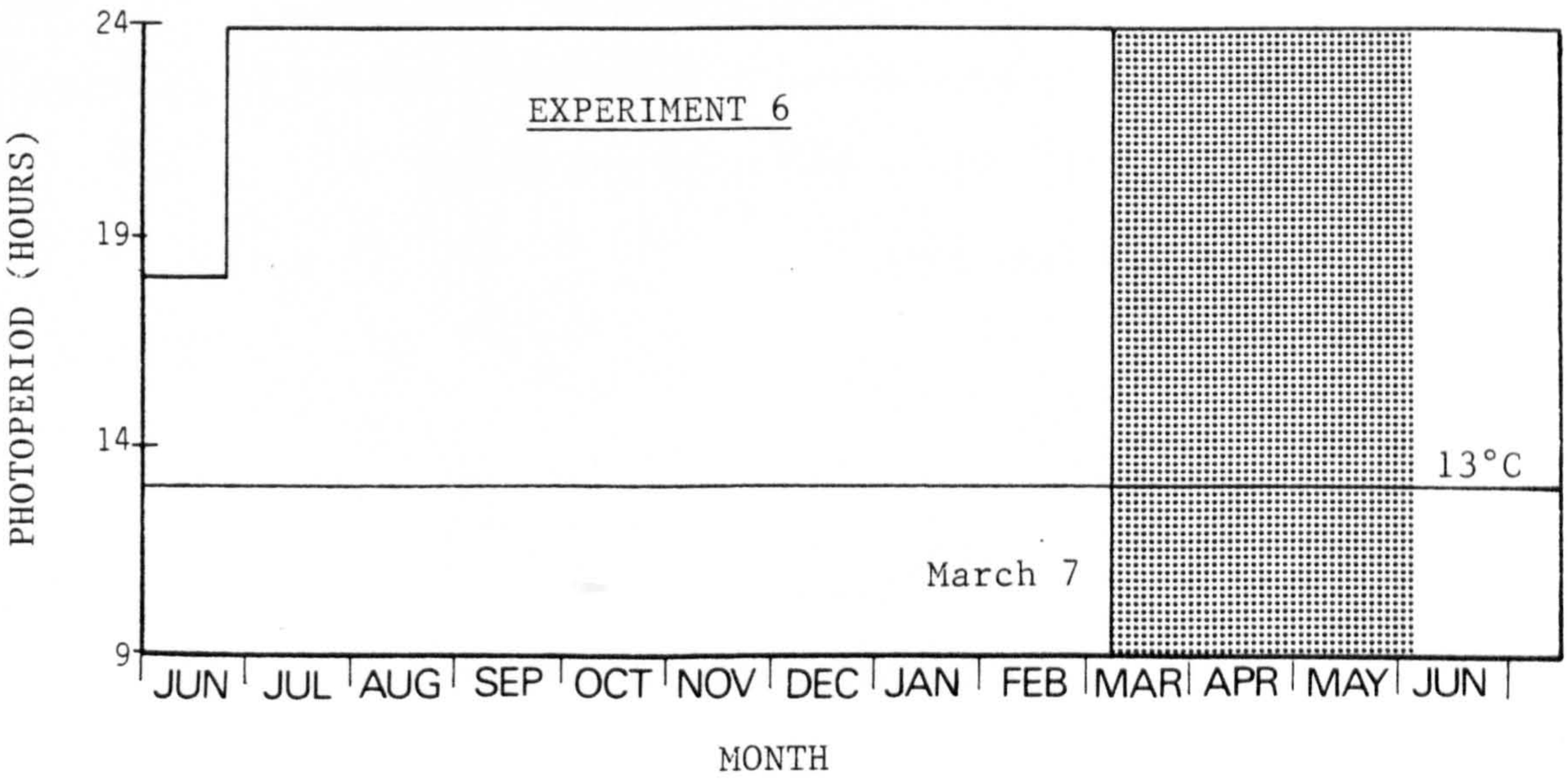

EXPERIMENT 4
(c)
PHOTOPERIOD (HOURS)
TEMPERATURE (°C)
20
15
10
5
0
20
40
60
80
100
120
140
160
180
200
220
DAYS

FIGURE 36 Experiment 5 : The photoperiod was maintained at 18.5 hours from the previous spawning. After a change in daylength from 18.5 to 14 hours the fish were maintained on a simulated natural photoperiod. Temperature was maintained close to 13°C (range 11 to 15°C): spawning period (stippled).

FIGURE 37 Experiment 6 : The fish were maintained on a constant photoperiod (24 hours light). Temperature was maintained close to 13°C (range 11 to 15°C): spawning period (stippled).

FIGURE 38 Experiment 7 : The fish were maintained on a constant photoperiod (18 hours light). Temperature was maintained close to 13°C (range 11 to 15°C): spawning period (stippled).


EXPERIMENT 5
PHOTOPERIOD (HOURS)
20
15
10
5
13°C
0
20
40
60
80
100
120
140
160
180
200
220
DAYS


EXPERIMENT 6
PHOTOPERIOD (HOURS)
24
19
14
9
13°C
March 7
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
MONTH

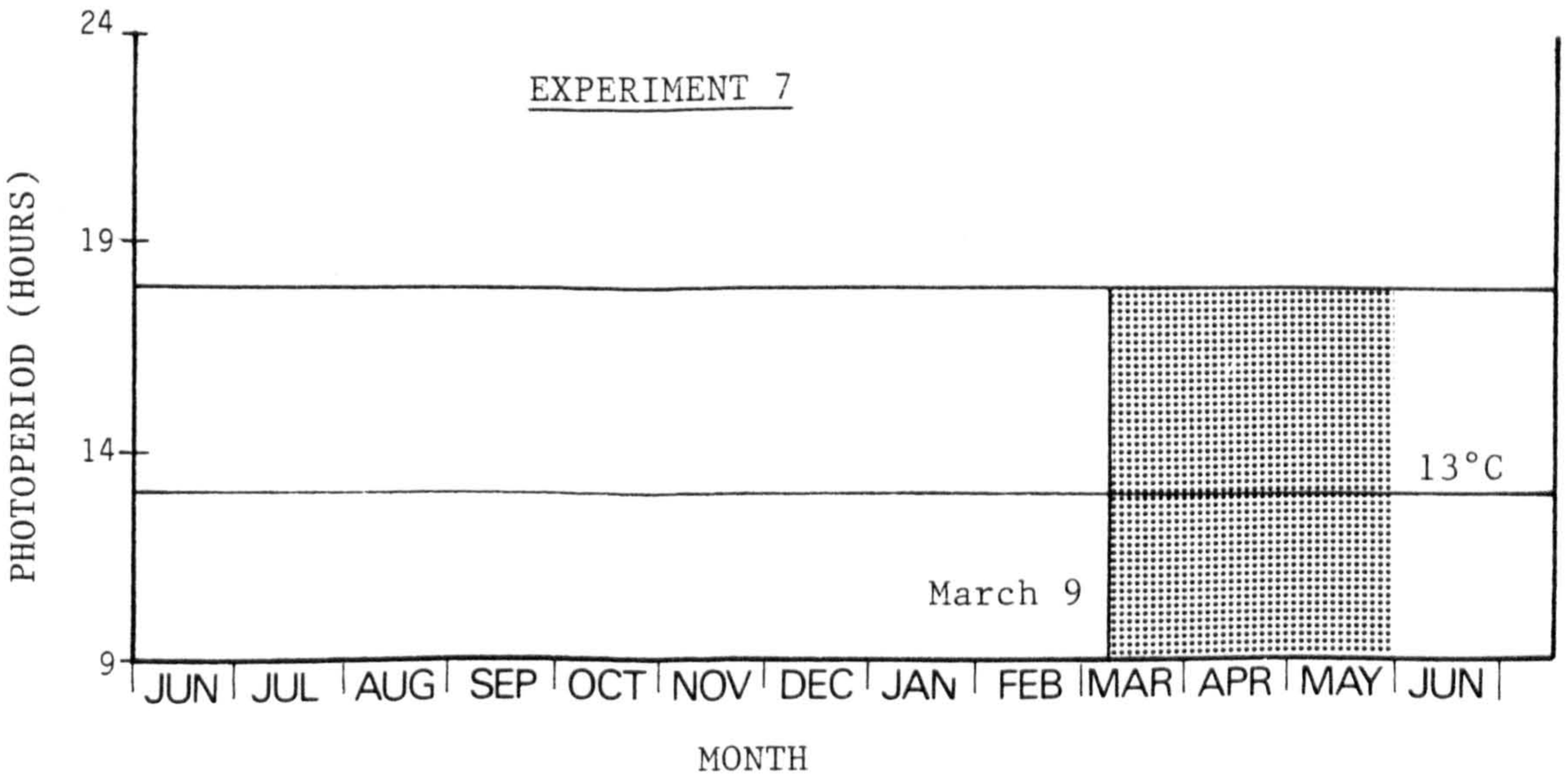

EXPERIMENT 7
PHOTOPERIOD (HOURS)
24
19
14
9
13°C
March 9
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
MONTH

FIGURE 38 Experiment 8 : The fish were maintained from the previous spawning on 18.5 hours daylength. During December the fish were placed on a simulated natural cycle of photoperiod two months behind the normal photoperiod. Temperature was maintained close to 13°C (range 11 to 15°C) : spawning period (stippled).

FIGURE 39 Mean monthly gonadosomatic index for females, Solea solea

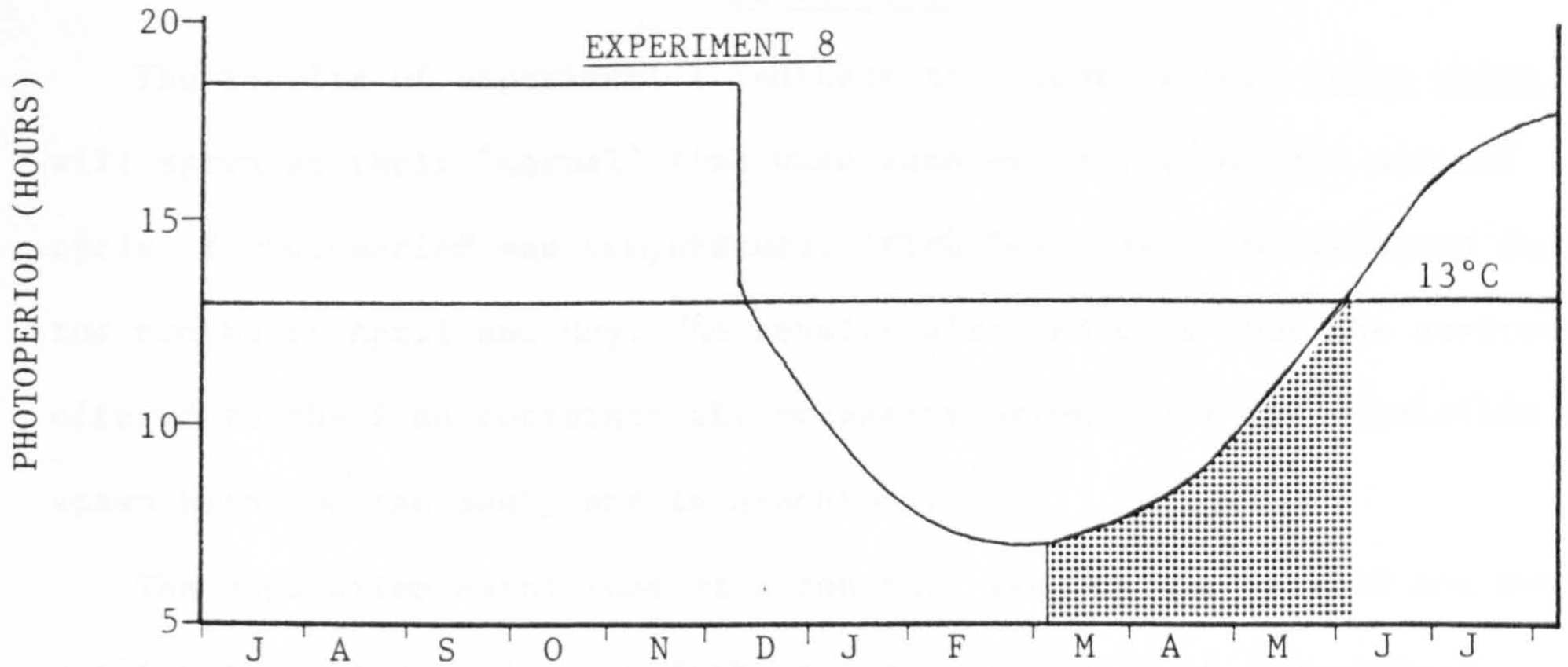
One SE (0.95) above and below the mean.

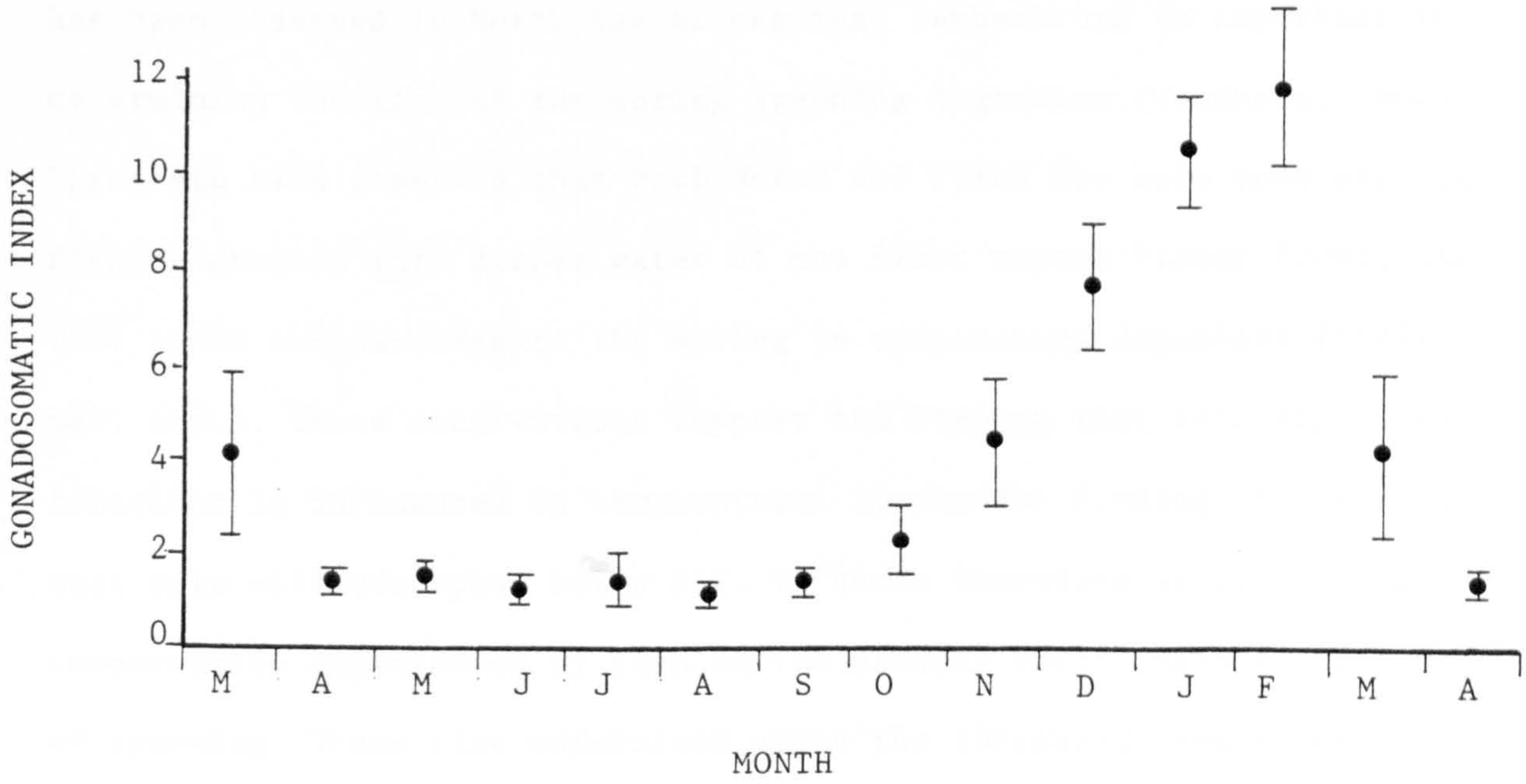
(from Deniel, 1981)

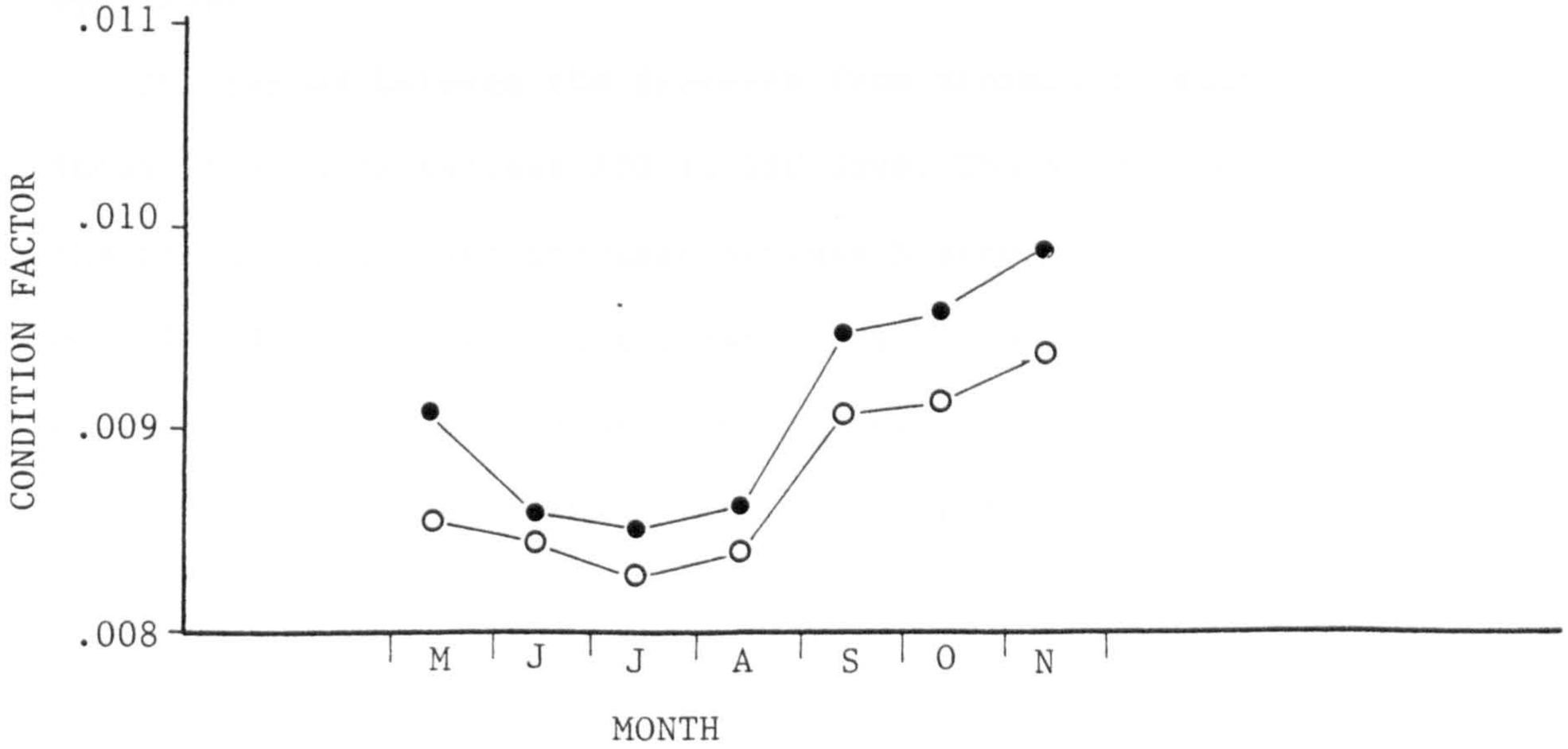
FIGURE 40 Average condition factor for females, Solea solea.

From 1968 to 1973, black dots; from 1963 to 1968 white dots.

(from de Veen, 1976)


EXPERIMENT 8
PHOTOPERIOD (HOURS)
20
15
10
5
13°C
J A S O N D J F M A M J J


GONADOSOMATIC INDEX
12
10
8
6
4
2
0
M A M J J A S O N D J F M A
MONTH


CONDITION FACTOR
.011
.010
.009
.008
M J J A S O N
MONTH

Discussion

The results of experiment 1 indicate that captive sole Solea solea, will spawn at their 'normal' time when exposed to a simulated natural cycle of photoperiod and temperature. Irish Sea sole normally spawn during the months of April and May. The results also indicate that the environment offered to the fish contained the necessary stimuli for the population to spawn both spontaneously and in synchrony.

The population maintained at a constant temperature spawned one month earlier (experiment 2), than fish on a natural cycle of temperature. This suggests a causal effect of temperature on the time of spawning. It has been observed in North Sea stocks that temperature is important in determining the time of the spring spawning migration (Woodhead, 1964). Fishermen have observed that both North and Irish Sea sole move off the fishing grounds into deeper water at the first severe winter frost, and that their reappearance in the spring is temperature dependent (Finlay, per. com.). These observations support the finding that sole migratory behaviour is influenced by temperature. It was the finding of this study that sole will not spawn below 8°C. It seems therefore as if the low temperatures experienced by fish on the natural cycle inhibit the onset of spawning. Those fish maintained above the threshold temperature of 8°C suffered no inhibition, and appeared to proceed straight to ovulation and spawning.

The period between the increase from minimal to maximal gonadosomatic index in sole is between 150 to 180 days. The gonadosomatic index shows the first significant increase between September and October (de Veen, 1976; Deniel, 1981). The earliest spawning period appears to be that of the Atlantic sole which extends from the end of February (Girin, 1976); this is very similar to the spawning time of the population in experiment 2. The results of experiments 3 and 4 indicate that the period between cue and spawning is at most 170 to 180 days and is within the above range.

The results from experiment 5 indicate that the period between the photostimulatory cue and spawning is shorter, at 162 days. For a population of sole to begin spawning at the end of February, the cue would need to be applied around the time of the autumnal equinox on 21 September.

In the photoperiod experiments, daylength during mid to late September lay in the range 13 - 15 hours, which is within the range of daylengths experienced at the autumnal equinox. These daylengths are also represented in experiment 4. The general indication is therefore that sole cannot enter into exogenous vitellogenesis in advance of a photostimulatory cue,which may be a fixed photoperiod, probably in the region of 13 - 15 hours.

Sole recruited from the wild and placed on continuous light (LL), and 'summer' (18.5L/5.5D), began spawning during early March of the following year. The timing was equivalent to experiment 2, where the population was maintained on a natural cycle of photoperiod. The only difference lay in the duration of the spawning period; the population in experiment 2 spawned over 4 - 6 weeks (normal), whereas the fish on continuous light spawned over several months. It appears therefore as if photoperiod acts by somehow initiating a particular phase of the reproductive cycle, in this case exogenous vitellogenesis, and synchronising this stage of development within the breeding population.

In the absence of the necessary proximal cue, the reproductive cycle is completed successfully (in captivity), but spawning synchrony within the population is affected. The beginning of spawning for populations maintained on continuous photoperiod and a natural photoperiod were very similar. That sole can achieve this precision in the absence of any cue, suggests an endogenous rhythm, possibly circannual.

The reason for the large spread in the spawning period is not clear. It seems reasonable to assume that there will be individual variation

in the timing of the decision to proceed with the next phase of the reproductive cycle (in the absence of a cue). In birds and mammals there is evidence to suggest that maintaining experimental animals under constant photoperiods leads to the expression of the 'free-running' period [τ] of an endogenous circannual rhythm (Saunders, 1977). The evidence also indicates considerable individual variation in the free-running (unentrained) period within populations. It may be therefore that the large spread in spawning period can be attributed to this. In the absence of a photo-stimulatory cue sole start the next physiological stage of their reproductive cycle spontaneously, possibly under the influence of an endogenous circannual rhythm.

The cummulative effects of asynchronous reproduction can best be illustrated by reference to a population of sole which spawned in captivity over three seasons. The fish were recruited from the wild, and had been exposed to a random series of photoperiods involving long periods on 18 hours light. The duration of the spawning period rose from six weeks to six months over the three reproductive seasons.

The results suggest a finite period when the reproductive system is responsive to photoperiod. Under a natural photoperiod cycle the effective cue appears to occur during late September. The sudden change in photo-period at the phase change in experiments 3 and 5 presumably exposed the fish to the effective photoperiod, and suggests that the period of responsiveness for some fish may extend from August. It may be therefore that the reason for a delayed cue in the natural situation is to allow more time for fish to recover physiologically from the previous spawning and possibly reach some limiting threshold of condition (Reshetnikov et al, 1970). Support for this suggestion comes from the increase in condition factor which occurs between August and September in wild populations (de Veen, 1976).

The extent of the responsive period is unknown but it may be

short. A continuous photoperiod is unlikely to provide the system with a cue, and it seems reasonable to assume therefore, that a long period of responsiveness should have resulted in delayed spawning in experiments 6,7 and 8. The fact that the populations in these experiments began spawning at the normal time suggests that the period might end during early October.

Additional evidence that the period of responsiveness is finite comes from experiment 8. The attempt to delay spawning by witholding a phase change until six months before the target spawning date of June, failed. Fish had obviously progressed with their reproductive cycle in the absence of a cue. The successful completion of the reproductive cycle was not inhibited by the sudden change in photoperiod, or the fact that spawning continued during a winter photoperiod. The results suggest that sole are refractory to photostimulation during the larger part of their reproductive cycle.

It is well established in vertebrates, that a natural cycle of photoperiod entrains circadian and circannual rhythms (Saunders, 1977). The entrainment ensures a circadian period which is close to 24 hours and a circannual period of 365 days. In the absence of a daily input of photoperiod information the 'free-running' period is expressed; the free-running circannual period in rodents varied from 44 - 59 weeks (Heller & Poulson, 1970). The evidence from this study suggests that sole possess an endogenous circannual rhythm. Although this rhythm probably influences the natural progression of the reproductive cycle in an individual, it does not appear to control the timing of spawning, which is under the influence of temperature.

During the months after spawning the breeding population can be divided roughly, into three groups: post spawning fish representing fish of different ages, new recruits, and fish which missed the previous spawning or cycle. Each of these groups have to attain a common synchrony

if they are to spawn successfully. The most appropriate time for synchronisation would appear to be the onset of exogenous vitellogenesis which is both the longest and most metabolicaly expensive phase of the reproductive cycle.

CHAPTER 3

Environmental Control Experiments

Summary

Temperatures less than 8°C appeared to inhibit the onset of ovulation and spawning in Solea solea and might be the reason for differences in spawning time throughout the range of the species. The spawning period is therefore ultimately determined by the sea temperature.

The onset of exogenous vitellogenesis might be cued by a specific photoperiod around the time of the autumnal equinox. The reproductive cycle of individuals within the breeding population would be synchronised as a result.

In the absence of a natural cycle of daylength the duration of the spawning period in captive sole increased beyond the normal time. This was probably due to the expression of the free-running period of a circannual rhythm.

Sole naturally spawn during the spring but they also spawn during winter photoperiods and the progression of exogenous vitellogenesis was unaffected by sudden changes in daylength. This suggests that the reproductive system is refractory to photostimulation for long periods. Sole could be made to spawn over one month in advance of the natural time by photostimulation in August; suggesting that some fish are photosensitive in advance of the natural cue.

The evidence suggests that a finite period of photosensitivity exists during which the physiological phase of exogenous vitellogenesis can be initiated. The period of photosensitivity is possibly related to a sudden but precisely timed increase in the general condition of the fish.

CHAPTER 4

The Pineal Organ

Introduction

In teleost fishes the pineal organ arises as a hollow outgrowth from the diencephalon, in common with the retina (Flight, 1979). The adult pineal comprises an end-vesicle and stalk which may have a lumen in open communication with the third ventricle, and be partially or completely closed (reviewed in Vollrath, 1981). The absence of a central lumen appears to be characteristic of deep sea fish (Holmgren, 1959) and blind cave fish (Herwig, 1976). Practically nothing is known about the blood supply of the teleost pineal other than the work of Bhargava (1973) who showed that the capillaries do not penetrate the pineal parenchyma. The following cell types are generally present: photoreceptor cells, interstitial cells (ependymal), and neurones (Oksche, 1971; Vollrath, 1981).

Electrophysiological recordings from the teleost pineal organ have demonstrated that the organ is involved in light perception (Dodt, 1963). Illumination of the organ leads to an inhibition of spike activity (achromatic response) with a dark adapted threshold of inhibition in the rainbow trout Salmo irideus = Salmo gairdneri of 3×10^{-5} lm/m^2 (Morita, 1975), and a rarer chromatic response has been reported (Hanyu et al, 1977; Falcón & Meissl, 1981). The pineal organ of teleosts may therefore act as an indicator of dusk (Hanyu et al, 1977), or daylength, in common with amphibians (Hamasaki & Esserman, 1976). It may thereby exert an influence on circadian and circannual rhythms (Falcón & Meissl, 1981).

There is little doubt that the pineal organ mediates the effect of photoperiod on teleost physiology, but the mechanisms of the system are far from clear. The organ has been implicated in the regulation of gonadotrophin rhythms in teleosts (Peter, 1982), and other systems (de Vlaming & Olcese, 1981) including the entrainment of circadian and circannual rhythms (Kavaliers, 1982).

One substance in particular, the indoleamine melatonin, has been singled out as an important inhibitor of gonad development. Melatonin has been identified in the pineal organ of the Pacific salmon Oncorhynchus tschawytscha (Fenwick, 1970) but its presence is based mostly on the identification of its precursor enzyme hydroxy-indole methyltransferase (HIOMT). This enzyme, although indicative of melatonin formation, does not irrefutably imply this molecule's synthesis because it can catalyse the formation of several compounds in addition to melatonin (Quay, 1974). Moreover, the source of plasma melatonin in teleosts is open to question as it has been shown that the retina contains HIOMT and can synthesise melatonin (Gern et al, 1978). It is far from being accepted that melatonin is the most important pineal hormone as there are other indoles in the organ which can suppress gonadotrophin secretion equally effectively. In mammals, pineal peptides such as arginine vasotocin have been shown to be even more effective suppressors of gonadotrophin secretion than the indoleamines (Lincoln & Short, 1980). Recently, arginine vasotocin was demonstrated in the salmonid pineal organ (Holder et al, 1982).

Using cobalt chloride iontophoresis, the pineal nerve tract was traced to mesencephalic and diencephalic areas, including the parapineal organ (Hafeez & Zerihun, 1974). The neural organisation of the pineal in teleosts is not fully understood and the neural connection between the pineal organ and the brain has yet to be elucidated.

Photoperiod can modify the morphology of the pineal (reviewed in Hoffman, 1970) and more recently evidence has been found for both seasonal and diel changes in pineal ultrastructure in Carassius auratus, (McNulty, 1981b,1982). Concentrations of melatonin precursors, serotonin and HIOMT, in the pineal organ of teleosts show a diel rhythm of activity (Smith & Weber, 1976; Yates & Herbet, 1976; van Veen et al, 1982); yet so far there has been little ultrastructural evidence of endocrine activity.

The pineal organ has been implicated as a transducer of photoperiod

information. Although the evidence suggests a connection with many physiological systems, the common link between results is a relationship to a seasonally varying photoperiod. In particular, the function of the organ in teleosts may be related to the mediation of photoperiod on annual physiological rhythms (de Vlaming, 1982), and as such is relevant to this study. The pineal organ of Coregonus lavaretus has not previously been investigated. It is the aim of this study to describe the morphology and ultrastructure of its pineal organ, and to establish the presence of photoreceptors.

Materials and Methods

Histological technique

Pineals for histological examination were dissected from fish immediately after their removal from gill or seine nets. Ova were maintained in plastic trays with a constant flow of water until hatching. The yolk-sac larvae were transferred to aquaria and maintained under natural daylight. The chorion of an ovum was punctured before its being placed in fixative; after 2-3 days the embryo was removed and processed. Larvae and fry were anaesthetised in cold water (1°C), before immersion in fixative.

Fixation was in Bouin's aqueous fixative (Pantin, 1946) for 2 to 3 days, followed by dehydration in 30%, 50%, and 70% ethanol. Picric acid was washed out by repeated changes of 70% ethanol. The tissue was further dehydrated in a 2-methylpropan-2-ol (Tertiary butyl alcohol, TBA) series:

70% embedding alcohol (dist. water, TBA, 95% ethanol, 3:2:5)	2-4 hours.
85% embedding alcohol (dist. water, TBA, 95% ethanol, 3:7:10)	2-4 hours.
95% embedding alcohol (TBA, 95% ethanol, 11:9)	2-4 hours.
100% embedding alcohol (TBA, 100% ethanol, 1:1)	2-4 hours.
TBA, 3 changes	2 hours. 24 hours. 2 hours.
TBA, paraffin, 1:1	6 hours.
"Fibrowax" (Arnold B. Horwell, Ltd.), 3 changes	2 hours. 24 hours. 2 hours.

Sections were cut at 5 µm on a Leitz rotary microtome. Masson's Trichrome stain was used for general morphology of the pineal organ. Tissue was stained with Weigert'S iron haematoxylin and counterstained in ponceau-acid-fuchsin and light green (B.D.H. Handbook).

Electron microscopy

All adult fish used for electron microscopy were seine netted at dusk,

and only two fish were sampled at any one time. No anaesthetic was used, because of the delay factor and the unknown effect anaesthetic might have had on the tissue. The fish were decapitated and the dorsal part of the skull was removed with a scalpel. Usually, the pineal organ and part of the stalk remained in situ within the cranial fossa. Within one minute of removal from the seine net, the pineal organ was immersed in fixative.

Fixation was in 6% gluteraldehyde in 0.1 M cacodylate buffer at pH 7.2, without sucrose (Herwig, 1976). Some attempt was made to match the temperature of the fixative to that of the lake water. The tissue was immediately returned to the laboratory and maintained at constant temperature (4°C) for about one hour. At the end of this period, the pineal was dissected out and sectioned into small pieces (< 0.5mm^3) prior to post-fixation in 2% osmium tetroxide in 0.1 M cacodylate buffer at pH 7.2 for another hour; the tissue was rinsed several times in cacodylate buffer prior to post-fixation. Dehydration was performed with a graded series of ethanol followed by immersion in propylene oxide prior to embedding:

Dehydration	50% ethanol	10 minutes
	70% ethanol	10 minutes
	80% ethanol	10 minutes
	90% ethanol	10 minutes
	100% ethanol	20 minutes
		17-18 hours
		20 minutes
"Araldite" solvent	Propylene oxide	20 minutes
		20 minutes
		20 minutes
Embedding	Propylene oxide/resin 2:1	1 hour at 60°C
	Propylene oxide/resin 1:2	1 hour at 60°C
	"Araldite" resin	48 hours at 60°C

Microtoming and staining

After embedding, tissue was selected for sectioning by inspection with a Zeiss binocular dissecting microscope. Blocks were mounted on "Araldite" pegs and then trimmed. Final trimming of the block, which contained the tissue, was carried out with glass knives on an L.K.B.

Ultratome III. 1 μm sections were cut, and stained with methylene blue (Mullinger, 1964). These thick sections were used as indicators of position within the tissue, for the thin sectioning that followed.

Thin sections were picked up on copper grids coated with pyroxylin and evaporated carbon film. Sections were doublestained in uranyl acetate (Gibbons & Grimstone, 1960) for 90 minutes, followed by lead citrate (Reynolds, 1963) for 2 minutes. Sections were viewed in a Philips E.M. 301 operated at 60 kV. Electron micrographs were taken on Ilford EM 4 plates.

Scanning electron microscopy

Pineals were fixed as for transmission electron microscopy. The tissue was dehydrated in a graded series of ethanol and after three changes of absolute ethanol, was placed in absolute acetone. The tissue was then critical point dried and fixed to aluminium stubbs with double backed "Sellotape". The tissue was gold coated in a sputter coater and viewed in a Cambridge S600 stereoscan electron microscope.

Results

Light Microscopy

The pineal organ in Coregonus lavaretus arises as a dorsal extension of the diencephalon, and lies within a shallow cranial fossa (Plate 2). The adult pineal organ comprises a stalk, and an end-vesicle which is between 1.5 to 3.0mm in length and 0.9 to 1.5mm in width. In vivo the organ is surrounded by lipid deposits, which do not cover its dorsal surface. Meningeal melanophores occur between the ventral surface of the organ and the dorsal sac (Plate 3). The adult end-vesicle is a dorso-ventrally flattened structure, with extensive infolding of the wall (Plates 2,3). At its most anterior point it reaches forward to the velum transversum, which also marks the beginning of the paraphysis. The paraphysis arises as a dorsal extension of the telencephalon and is not well developed in this species (Plate 2).

The appearance of the pineal organ resembles the convoluted type described by Omura and Oguri (1969), which occurs in other salmonids. The dorsal parenchyma is similar in appearance to the ventral arrangement of cells but appears to be shallower (Plate 2). The end-vesicle contains an extensive lumen, which through the hollow stalk, is in open communication with the third ventricle; therefore cells bordering the lumen are bathed in cerebro-spinal fluid. The end-vesicle is contained within a connective tissue capsule, which is especially prominent in the external folds of the epithelia. Many blood vessels and capillaries occur in and around the connective tissue capsule, but none were seen to enter the pineal parenchyma (Plate 3). A large sinus is present ventral to the end-vesicle (Plate 2).

The pineal stalk originates from the wall of the third ventricle between the habenular commissure and the sub-commissural organ; it then lies alongside the dorsal sac (Plate 4). For most of its length the stalk is surrounded by folds of the dorsal sac.

The parenchyma of the pineal organ contains three main cell types: photoreceptor cells, interstitial cells, and neurones. Macrophage cells are regularly found throughout the lumen, and could be distinguished by darkly staining lysosomes in the cytoplasm (methylene blue stain).

Photoreceptor cells : The photoreceptor cells occurred close to the luminal aspect of the epithelium and possessed a nucleus of oval or round appearance (Plate 3). There appeared to be no regional differences in the presence of photoreceptor cells. Two processes may be seen: a basal process which can be of varying length, and which terminates within areas of neuropile; an apical process where the outer and inner segments project into the lumen (Plate 3). Outer segments can be clearly seen in tissue fixed with gluteraldehyde, and stained in methylene blue; they varied in appearance from straight to circular.

Interstitial cells : Interstitial cells or 'supporting' cells occurred throughout the parenchyma and are identified by their basal processes which extend to the basal lamina (Plate 6). The apical process is difficult to detect in Bouin fixed material, where it appears very thin. In gluteraldehyde fixed tissue the interstitial cells stain weakly in methylene blue in comparison to photoreceptor cells. The nuclei are of varying shapes: angular, round and elongate profiles.

Neurones : Nerve cells occurred throughout the epithelium but were relatively scarce. They appeared to be larger than the surrounding cells and both the nucleus and cytoplasm stained weakly in methylene blue (Plate 33). The nucleus was circular and possessed a darkly staining nucleolus; the main body was circular with a single process.

In the proximal region of the end-vesicle the basal epithelium was composed mainly of nerve tissue (Plate 7). In the upper regions of the stalk, cross sections revealed that the axons were arranged in bundles around the periphery, but within the basal lamina. In contrast to the numbers of unmyelinated axons, the presence of myelinated axons were

remarkably rare; the maximum number of myelinated axons in a cross-section was six. At some undetermined point in the upper to mid region of the stalk the nerve bundles combined to form the pineal tract (Plate 4). The greater part of the stalk was not examined by electron microscopy and therefore it was not established whether the tract runs outwith the basal lamina. The stalk joined the wall of the third ventricle between the habenular commissure and the sub-commissural organ (Plate 8), at which point the nerve tract continued on between the dorsal posterior commissure and the ventral sub-commissural organ (Plate 5).

Development : The pineal organ was evident in embryos at 48 days after fertilisation and was found to contain a narrow lumen. At 55 days after fertilisation (pre-hatch), the lumen was more extensive, but the anterior development is not well advanced; the stalk was still relatively undeveloped (Plate 8). The lumen of the stalk and end-vesicle can be seen in open communication with the third ventricle (Plate 8); the relationship to the habenular commissure and sub-commissural organ is clearly shown. After 74 days (immediately after hatching) the anterior development was well advanced and the end-vesicle lay immediately under the skull; no infolding of the walls had occurred. The stalk was well developed by 94 days and in transverse section can be seen entering the third ventricle (Plates 9,10). The stalk approaches the sub-commissural organ over the surface of the habenular commissure; to the left the parapineal organ is connected to the habenular commissure by a nerve tract (Plate 9). At the point where the stalk enters the ventricle its walls connect with the sub-commissural organ; the parapineal organ continues on the left (Plate 10). The parapineal organ was not observed in the adult but because of its small size and position it could easily have been overlooked.

Figure 42

A diagrammatic reconstruction of pineal epithelial organisation, from light and electron microscopy.

e - erythrocyte

dos - disintegrated outer segment

ic - interstitial cell

in - interstitial cell nucleus

ip - interstitial cell process (pvs)

ln - lateral neuropile

mm - meningeal melanophore

n - neuropile

nc - nerve cell

os - outer segment

pc - photoreceptor cell

pvs - peri-vascular space

Not to scale.

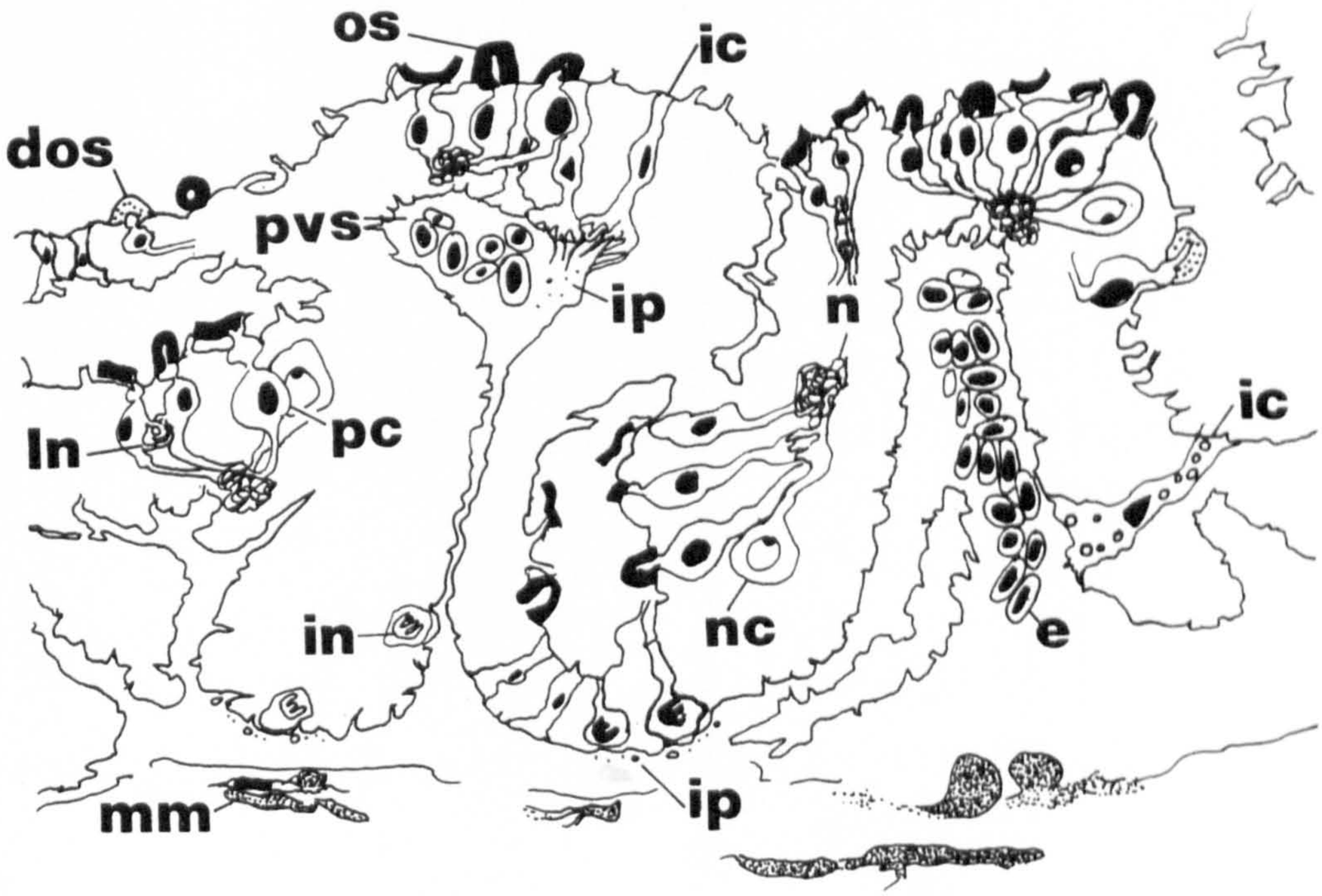

os
ic
dos
pvs
ip
n
ic
ln
pc
in
nc
e
mm
ip

Figure 43

Drawings of photoreceptor cell outer segments showing their structure; reconstructed from serial sections.

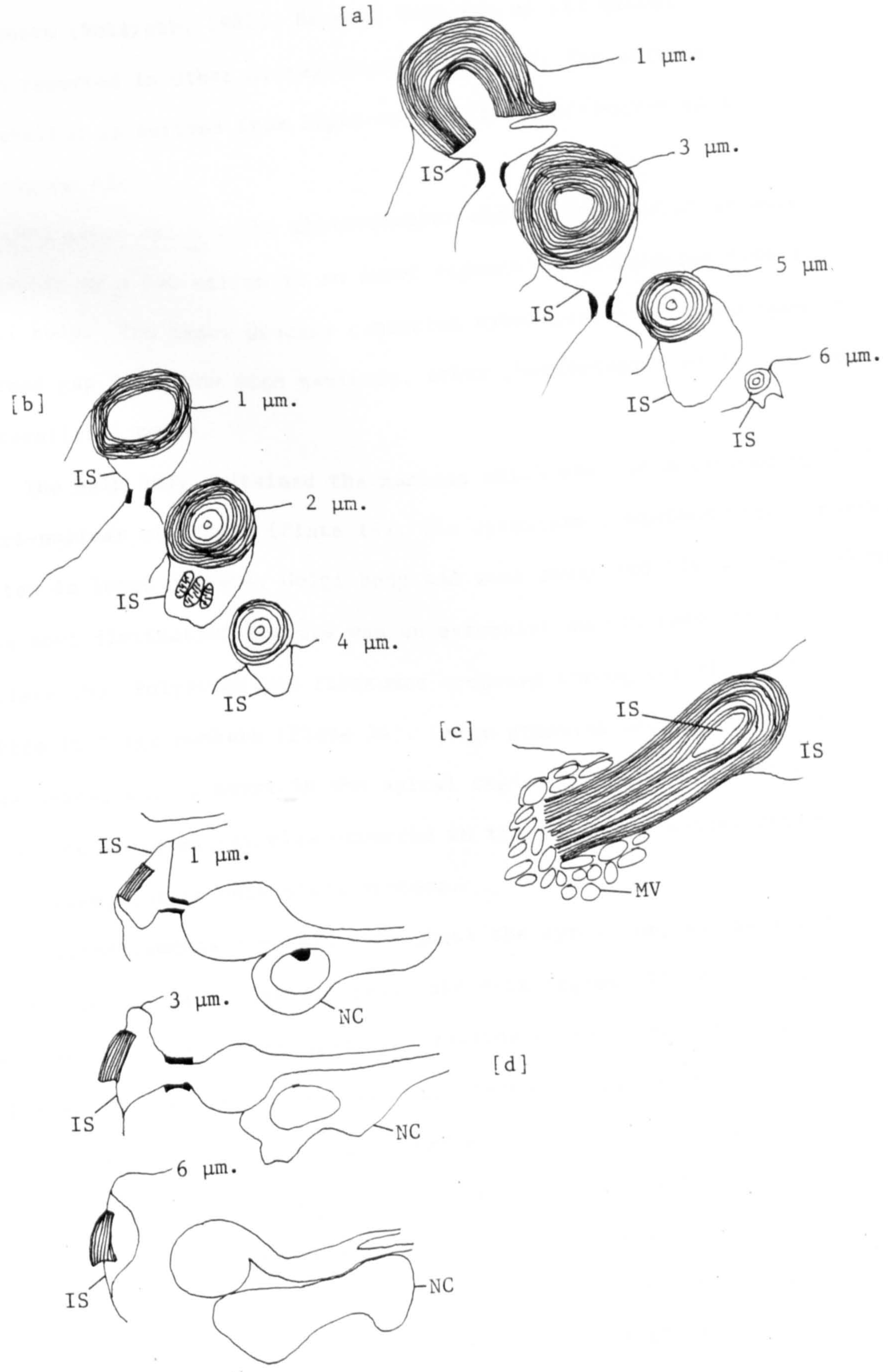
[a] The ends of the 'horse-shoe' appear to join, forming a circular profile which tapers to a point.
IS - inner segment

[b] An outer segment with a circular profile which terminates as a dome. The gaps between and within individual lamellae increase at the end of the segment.
IS - inner segment

[c] A long outer segment which has folded over on itself. This type is probably related to the long and narrow outer segments found in some scanning electron micrographs (Plate 42).
IS - inner segment
MV - microvilli

[d] A transverse section through a straight outer segment.
IS - inner segment
NC - nerve cell

NOT TO SCALE


[a]
1 µm.
3 µm.
5 µm.
6 µm.
IS
IS
IS
IS
[b]
1 µm.
2 µm.
4 µm.
IS
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IS
[c]
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IS
MV
[d]
IS
1 µm.
3 µm.
NC
IS
NC
6 µm.
IS
NC

Electron microscopy : The ultrastructure of the pineal organ in Coregonus lavaretus has the same basic pattern as previously described in other teleosts (Vollrath, 1981). Several features of its pineal organ have not been reported in other ultrastructural studies. The structure of the pineal epithelium as derived from light and electron microscopy is reconstructed in figure 42.

Photoreceptor cells : The photoreceptor cells consisted of an outer segment attached by a 9+0 cilium to an inner segment which extended from the main cell body. The basal process connected synaptically with neurones and formed gap junctions with neurones, other photoreceptor cells, and possibly interstitial cells.

The main body contained the nucleus which was characterised by distinct peri-nuclear cisternae (Plate 16). The cytoplasm contained mitochondria, often in large numbers, Golgi body and some rough endoplasmic reticulum; the most distinctive feature was an extensive smooth endoplasmic reticulum (Plate 16). Polysomes and ribosomes occurred throughout the cytoplasm, often in large numbers (Plate 14). Large granular bodies, possibly lysosomes, were present in the apical region of the cell (Plates 15,17). Small dense cored vesicles occurred in the basal and apical regions of the cytoplasm, but in very small numbers.

Microfilaments occurred throughout the cytoplasm, and were especially prominent in the following areas: the neck region (Plate 14), within the smooth endoplasmic reticulum, and running parallel to the cell axis (Plates 18,19), and in the terminal processes (Plate 35). Microtubules were present, but only in small numbers.

The cytoplasm of the photoreceptor cells was slightly electron dense and differed from interstitial cells and neurones which had relatively electron lucent cytoplasm (Plates 13,17). The degree of electron density between adjacent photoreceptor cells could differ (Plate 19).

The outer and inner segments extended into the lumen, and the inner

segments were bordered by the microvilli of the interstitial cells (Plates 13,15). There was little difference in appearance between the cytoplasm of the main cell body and the inner segment (Plates 14,16). The narrow neck region (isthmus) is characteristic of the photoreceptor cell and was connected to adjacent interstitial cells by a zonula adherens like junction (Plate 13).

Cross sections through outer segments show the lamellae arranged in a variety of shapes: mainly straight, horse-shoe and circular (Plates 12,13, 15). Analysis of serial sections suggests that the the three types of profile are simply cross sections, at different points, within a single outer segment (Fig. 43). Individual lamellae can merge (Plate 15); it may be therefore that the circular profiles are created when the ends of the segment meet. There is a possibility that the outer segments may be distorted during the fixing and embedding processes.

The stack of lamellae in the outer segment was created from folds of the cell membrane which in areas appeared to be open to the extracellular space. Serial sections revealed that the tissue on the periphery of the outer segment was tubular (Plate 13) and ran along its length. Individual outer segments could be up to 8 μm wide, 2.2 μm deep and 9 μm long; the number of lamellae ranged between 15 to 50. It proved difficult to obtain perfect transverse sections of both inner and outer segments in one field as the segments were often joined at an angle.

A few outer segments showed varying degrees of tubule formation, from partial to complete (Plates 14,21). The tubule formations occurred within outer segments which were otherwise well fixed (Plate 14), and it may be therefore that they represent an intermediate stage in the breakdown process.

Outer segments and 9+0 cilia were found in the embryonic pineal organ at 55 days after fertilisation (Plate 11). No areas of synaptic contact were observed and the interstitial cells had no apical microvilli. The

close proximity of the third ventricle which is continuous with the pineal lumen, indicates that the outer and inner segments are bathed in cerebro-spinal fluid (Plate 8).

The basal processes of the photoreceptor cells converged towards neuropile areas which occurred throughout the mid and basal epithelium. Synaptic ribbons were a characteristic feature of the terminal processes; the synaptic connections however are difficult to trace. Post-synaptic sites showed increased electron density immediately behind the cell membrane (Plates 38,39). The synaptic ribbons were separated from the pre-synaptic membrane by an area of increased electron density (Plate 38.).

The synaptic ribbons were associated with vesicles which varied in appearance from clear to slightly electron dense (Plates 38,40). The diameter of the vesicles ranged between 30 to 40nm with a mean value of 33nm . Between adjacent terminal regions, differences could be found in the distribution and number of vesicles. The process in plate 40 is full of vesicles whereas there are very few vesicles in plate 39. Besides vesicles the terminal regions contained straight, or slightly curved synaptic ribbons orientated obliquely or parallel to the cell membrane; rarely, multi-vesicular bodies were found (Plate 35).

The neuropile when viewed in sagittal section was composed of a central nerve process, usually a dendritic spine, enclosed by a photoreceptor cell basal process (Plate 35). In transverse section this arrangement was represented by the circular profile of the nerve process surrounded by the 'halo' profile of the photoreceptor process. Neuropile can be very complicated with the cell processes arranged in a tortuous fashion. The processes of seperate photoreceptor cells were layered around a central nerve process or processes (Plate 36). Interpretation of transverse sections is difficult and further handicapped by the presence of interstitial cell processes (Plate 35.).

A feature of the neuropile areas were specialised membrane contacts

which resembled gap junctions (Plate 20). These junctions occurred in neuropile which also contained synaptic ribbons (Plates 36,39); gap junctions occurred most frequently where the lateral processes of several photoreceptor cells intertwine [lateral neuropile] (Plates 18,19,20). The processes were mainly derived from photoreceptor cells but occasionally a process originated from an unidentified cell (Plate 19). A possibility exists therefore that nerve processes or interstitial cell processes might be associated with the lateral neuropile. Tight or gap junctions occurred between nerve cell processes and photoreceptor cell processes (Plate 39).

Interstitial cells : Interstitial or 'supporting' cells occurred throughout the pineal epithelium in association with photoreceptor cells; unlike neurones and photoreceptor cells the interstitial cells extended from the lumen to the basal lamina. The cells had a border of microvilli which extended into the lumen and basal processes which extended into the peri-vascular space. The main basal process of the cell subdivided into smaller processes which formed a tortuous network in the region of the basal epithelium. Individual processes were connected by a zonula adherens (desmosome with tonofilaments) junction (Plate 29).

In contrast to the photoreceptor cells there was little or no dilation of the peri-nuclear cisternae. The cytoplasm contained the dilated cisternae of smooth endoplasmic reticulum, Golgi apparatus and a loose network of microfilaments (Plates 26,35). Ribosomes and polysomes occurred throughout the cell. Dense cored vesicles with diameters which ranged from 130 - 370nm occurred throughout the cell; they did not occur in large quantities (Plate 16).

Occasionally the apical region of the cell was found to be 'sheet' like and to contain the dictyosomes of Golgi apparatus (Plate 17). The cytoplasm was relatively empty and contained few ribosomes or polysomes. Adjacent to the Golgi apparatus (Plate 17) there were several organelles

with partial double membranes; suggesting that they were mitochondria.

The cristae of interstitial cell mitochondria were not well developed (Plates 12,16,25), which might be due to poor fixation. However, both 'normal' ellipsoidal mitochondria with cristae and the more dilated type occurred on the same section (Plate 27). The mitochondria of both neurones and photoreceptor cells resembled an ellipsoid shape and contained cristae (Plates 15,34). Although the other cell organelles within the cytoplasm appeared to be well fixed, the large mitochondria were a characteristic feature of interstitial cells (Plate 28).

The degenerated lamellae of photoreceptor cell outer segments were often associated with the microvilli in the apical region of the cell (Plate 21). Interstitial cells might therefore be involved in the breakdown of the outer segment (Plate 16).

The nuclei of interstitial cells showed considerable plasticity, and were found in a variety of shapes. The appearance of the nucleus may be related to cell activity. Cells with an indented nucleus contained the cisternae of smooth and rough endoplasmic reticulum, large mitochondria, and the cytoplasm was full of ribosomes and polysomes (Plates 27,28). Cells with angular or elongate nuclei were characterised by the dilated cisternae of smooth endoplasmic reticulum, Golgi apparatus and few ribosomes or polysomes. The cells with indented nuclei were only found close to the basal lamina in the external folds of the epithelium (Fig. 42) They possessed a nucleus with either normal electron density or high electron density (Plate 27).

Nerve and interstitial cell cytoplasm was relatively electron lucent, which made positive identification of the cell processes in the basal epithelium difficult. Photoreceptor cells also occurred close to the basal lamina, but the processes were easily identified. In general the majority of cell processes bordering the basal lamina belonged to interstitial cells.

Between the basal lamina of the pineal epithelium and the basal

lamina of the endothelial cells, a peri-vascular space containing collagen was present. The basal lamina surrounding the pineal organ was more developed than the one surrounding the endothelial cells (Plate 22).

The interstitial cells gave rise to processes which were contained within the basal lamina, and extended into the peri-vascular space. The space between the cell membrane and the basal lamina contained small vesicles (Plates 22,27); their diameter ranged between 25 - 50nm with a mean value of 37nm. The majority did not have an electron dense interior but a few of the larger vesicles contained an electron dense core (Plates 23,24). The basal lamina and vesicles were observed to extend up to 2 μm beyond the cell process (Plate 24). Transverse sections through the basal lamina and vesicles or the basal lamina alone in the peri-vascular space were commonly found (Plates 22,32).

Electron dense material within large vesicles, and tubular structures with an electron dense appearance were found in cell processes close to the basal lamina (Plates 31,32). Clear vesicles were observed within an electron dense vesicle (Plate 31). However, the cell type involved was not positively identified.

The processes which interface with the basal lamina were characterised by hemidesmosome specialisations of the cell membrane (Plate 29). Ribosomes, polysomes, the cisternae of smooth endoplasmic reticulum, and small dense cored vesicles occurred throughout these processes.

A feature of the cell processes in the basal epithelium was the presence of clear or slightly electron dense vesicles which appeared to be derived from the cisternae of smooth endoplasmic reticulum. The vesicles belonged to a different size class from the photoreceptor synaptic vesicles and the small vesicles associated with the basal lamina. The diameters ranged between 54 - 100nm with a mean value of 71nm. The vesicles connected with the cell membrane (Plate 29), and were found in groups (Plate 30).

Neurones : The nerve cells have a large circular nucleus with a prominent

nucleolus (Plate 33). The main cell bodies occurred infrequently throughout the epithelium but tended to lie near the lumen. The cell body contained Golgi apparatus, dense cored vesicles, both rough and smooth endoplasmic reticulum, polysomes and ribosomes (Plate 34). The nerve cell mitochondria had electron dense interiors (Plates 34,37).

The nerve cell processes contained multi-vesicular bodies, normally in association with areas of neuropile (Plates 35,36,39); they were also found in photoreceptor basal processes.

In the neuropile formations, the processes of nerve cells were often difficult to identify with certainty mainly because of a lack of identifiable features. When neuropile was viewed in a suitable plane the dendritic spines were found to occupy a central position surrounded by photoreceptor cell processes (Plates 35,37).

Dendrites and dendritic spines were identified by the presence of an electron lucent cytoplasm, parallel arrays of microtubules, and electron dense mitochondria (Plate 37). The synaptic sites were identified on the basis of the increased electron density of the area behind the post-synaptic membrane (Plates 38,39).

Included in the cytoplasm of the main body were a number of dense cored vesicles of mixed size (180 - 400nm) which showed no apparent relationship to the Golgi apparatus. On a few occasions electron dense material was observed in the intercellular space between dendrites and photoreceptor cells (Plate 37).

The confused nature of the pineal epithelium made it virtually impossible to follow nerve cell processes for any distance without the use of specialised techniques which were outwith the scope of this study. The proximal region of the end-vesicle contained large nerve tracts (Plate 7) which converged in the peripheral region of the upper stalk. Myelinated axons were observed in this area but only in small numbers. The largest number seen in any one section was six. No attempt was made

to examine the entire stalk by electron microscopy.

Scanning electron microscopy : The pineal lumen was characterised by the presence of inner and outer segments of photoreceptor cells and the microvilli of interstitial cells.

The intraluminal septa are shown in Plate 41 and confirm the arrangement of tissue shown by light microscopy (Plate 3). The network of microvilli in which the inner and outer segments lie is shown in Plate 42 and confirms the general view of cells bordering the lumen shown in Plate 12. The long outer segments found in scanning electron micrographs (Plates 41,42) also appear in electron micrographs as illustrated in figure 43 c.

Discussion

The pineal organ of Coregonus lavaretus contains photoreceptor cells with well developed outer segments and basal processes which make synaptic contacts with nerve cells. These findings are in general agreement with other morphological (Vollrath, 1981) and electrophysiological studies (Morita, 1975; Hanyu et al, 1977) which have shown that the teleost pineal functions as a photoreceptive organ.

The cranial fossa, dorsal to the pineal organ, appears to be an adaptation facilitating the passage of light. The semi-transparent area over the pineal allowed the pale outline of the optic tectum and telencephalon to be observed in live fish recovered from nets. Severing the spinal cord resulted in dispersion of pigment in the skin above the cranium making it opaque. A discussion of the control processes involved in melanophore dispersion can be found in Bhargava (1973) and Nilsson et al (1983). Although penetration of light through the skin and skull was not measured, it has been investigated in other species. It was found in the pike Esox lucius that the skin and skull did not attenuate the incident light by more than 0.5 log units (Falcón & Meissl, 1981), Little information is available on the spectral absorbance of the tissue covering the organ in teleosts.

Relatively little is known about the shape of the outer segments in teleosts. Scanning and transmission electron microscopy studies on a few species suggest that there may be three (Bergmann, 1971) or four types (Ueck et al, 1978). It has been speculated that the variable outer segment morphology may represent a cyclical renewal process of the outer segments (Herwig, 1976). This explains some of the findings in the powan pineal but analysis of serial sections suggests that there may be two main types. It was found that the three main profiles, straight, horseshoe and circular represented different cross-sections through a single outer segment. Individual lamellae appear to be able to merge, which suggests

that the ends of the horseshoe can join to form a circular profile. The resulting shape is a stack of lamellae which curve to form a domed end. The most common form of outer segment is a simple stack of lamellae, which appears to predominate in the scanning electron micrographs. It is not known whether the circular profile of the outer segments is an artefact of the fixation process.

The sites of synaptic transmission in teleost retinal photoreceptors are characterised by the presence of synaptic ribbons (Vollrath, 1981). In pineal tissue, although the ribbons are commonly found, the synaptic connections have proved difficult to find in many species. Recently, it has been shown that the synaptic relationship between photoreceptors and neurones in Salvelinus fontinalis and Salmo gairdneri is influenced by photoperiod (Omura and Ali, 1980). In Carassius auratus, diel rhythms in the concentration, length and distance from the plasma membrane, of synaptic ribbons were induced by photoperiod and maintained in constant darkness (McNulty,1981b). In powan, cells showing the post synaptic specialisations are nerve cells, indicating that photoreceptor cells in this species do synapse with neurones.

Two types of synapse between photoreceptor cell processes and neurones have been reported in Salmo gairdneri (Omura, 1979) and are influenced by photoperiod (Omura and Ali, 1980). Only one type of chemical synapse was observed in powan, and no attempt was made to differentiate between nerve cells.

Specialised contacts between photoreceptor cells, which resemble gap junctions have been reported in Astyanax mexicanus (Herwig, 1976), Carassius auratus (McNulty,1981a), Gasterosteus aculeatus (van Veen et al, 1980), Esox lucius (Falcón, 1979), and others. In powan it can only be said that such junctions occur between photoreceptor cells and possibly another cell type. Gap junctions have been associated with electrotonic conduction in vertebrates, and McNulty(1981a) has speculated that they

might integrate the activity of a large number of sensory cells to a single nerve cell. Although the functional significance of these specialised membrane contacts, in the teleost pineal, are still speculative they emphasize the complexity of interactions between the different cell types.

In the present study no morphological evidence was found for a membrane bound granular secretory activity similar to the one described in the pineal organ of birds (Ueck, 1974) and reptiles (Collin, 1971). Previous studies on teleost pineals have consistently failed to demonstrate evidence for a secretory function. However, it has been established that the cells contain the indoleamines serotonin and melatonin (Fenwick, 1970; Hafeez & Zerihun, 1976) and the precursor enzymes involved in their synthesis (Hafeez & Quay, 1970; Smith & Weber, 1976).

The cytoplasm of photoreceptor and interstitial cells with their Golgi bodies, microfilament bundles, and extensive endoplasmic reticulum is suggestive of a secretory role. Dense cored vesicles were found throughout the cytoplasm of both cell types but never in any quantity. Tissue for electron microscopy was obtained at dusk only and the possibility of diel and seasonal variation in the activity of pineal cells was not investigated. A diel cycle of activity is already established (Smith & Weber, 1976; Yates & Herbet, 1976; van Veen et al, 1982) and recently seasonal changes in pineal ultrastructure have been demonstrated in Carassius auratus, (McNulty, 1982). Further investigation is required therefore before it can be said conclusively that evidence for a membrane bound secretory activity is not present.

The interstitial cells of the teleost pineal have received little attention and consequently their function remains unclear. The presence of membranous inclusion bodies resembling outer segment material supports the suggestion of Herwig (1976) that they may be involved in the breakdown of the outer segments.

Cell processes which project into the perivascular space and contain predominately clear vesicles are a characteristic feature of some mammalian pinealocytes and have been associated with a secretory role (Vollrath, 1981). Processes which are within the basal lamina and also without have both been reported (Wartenberg, 1968). The finding of this study, that the interstitial cells have processes which extend into the perivascular space has not previously been reported in teleosts. The content and function of the small vesicles is unknown but their association with the basal lamina suggests a possible role in its assembly. The processes contain ribosomes, polysomes, dense cored vesicles and hemi-desmosomes but their function is unknown. However, they do significantly increase the surface area of the cell and emphasise the active appearance of this cell type.

Clear vesicles were also found in the processes of cells bordering or close to the basal lamina; in some cases the vesicles appear to be associated with the cisternae of smooth endoplasmic reticulum. The very electron lucent and relatively empty cytoplasm is not characteristic of interstitial cells which raises doubt over the identity of the cell. Cell processes in this area are tortuously arranged and caution is required in interpretation. The vesicles are of a different size range to the clear vesicles in photoreceptor cell processes and their content and function are unknown. Further work is required to establish the identity of these processes and to eliminate the possibility that the vesicles are artefacts. No similar findings have previously been reported in teleosts.

Ultrastructural features of pineal nerve cells in powan suggest that they are metabolically very active. The main cell body contains prominent Golgi bodies associated with both clear and dense cored vesicles, rough and smooth endoplasmic reticulum, mitochondria and polysomes. Dense cored vesicles were described in Carassius auratus (McNulty, 1981a), Carassius

gibelio (Ohba et al, 1979), and Esox lucius (Falcón & Mocquard, 1979); the contents of the vesicles and therefore their significance is unknown.

Single dendrites appear to contact several photoreceptor cell processes and the associated axons contain large numbers of mitochondria suggesting activity. Using the acetylcholinesterase method it has been demonstrated in Carassius auratus that up to 100 photoreceptor cells can be related to one nerve cell (Ohba et al, 1979). In any future study the hypothesis of McNulty and Nafpaktitis (1977), that the convergence ratio of sensory cells to neurones may act as an indicator of photo-sensitivity, could be useful in assessing the ecological significance of the powan pineal.

It was outwith the scope of this study to consider the nervous organisation of the pineal in any detail and the reader is referred to the following reviews: Ueck (1979), and Vollrath (1981). Most of the fibres in the pineal stalk form a nerve tract which was traced into the area between the posterior commissure and the sub-commissural organ; this is in agreement with other studies (Hafeez, 1971; Hoffman, 1970; Bhargava, 1973; and others). Myelinated fibres were found to be rare in the upper region of the stalk which agrees with the findings in Salmo gairdneri (Omura, 1979). Omura also found that although myelinated fibres were undetectable throughout most of the stalk they did appear after the habenular commissure and increase in numbers towards the posterior commissure. He suggests that myelinated fibres in the latter region are efferent and that those in the upper stalk, in common with unmyelinated fibres, are afferent. The functional significance of the myelinated fibres in teleosts is unknown.

The presence of well developed outer segments in the embryo of the powan suggests that the pineal may be active at an early stage of development. This period remains unexplored in teleosts but the presence of photoreceptor cells may have relevance to a possible role of the pineal

in the entrainment of circadian and circannual endogenous rhythms. The fundamental importance of such rhythms to the temporal co-ordination of animals suggests that entrainment might be expected to operate at an early stage of development.

The pineal organ of the powan appears to be equipped to function as a photoreceptor as its position within the cranial fossa suggests (Plate 2). The extensive infolding of the epithelium and the luminal networks provide a very large surface area for such a small organ. When combined with the network of blood vessels and capillaries it suggests a possible secretory role. The general arrangement of the cells indicates a complex organisation. Although no evidence was found for membrane-bound secretory activity, such a possibility cannot be ruled out on the basis of this study which took no account of diel and seasonal variation in activity. Moreover, it may not be the primary function of the cells to store large amounts of secretory material which could be released on production.

Fluoresent histochemical techniques may be of value in indicating the general presence of indoleamines within the tissue but the tortuous arrangement of cell processes would give such methods limited specificity. Any extension of this study will require the use of autoradiographic techniques for the demonstration of indoleamines (Hafeez & Zerihun, 1976) and histochemical enzyme techniques for the demonstration of nerve cells (Wake, 1973; Korf, 1974).

CHAPTER 4

The Pineal Organ

Summary

The pineal organ of the powan Coregonus lavaretus (L.) was investigated by light and electron microscopy. The organ in the adult lies within a shallow cranial fossa which appears to be an adaptation facilitating the passage of light. The end-vesicle is very convoluted and contained within a connective tissue capsule. Capillaries run through the external folds of the pineal epithelium but do not enter the organ.

Three main cell types were found: photoreceptor cells, interstitial cells and neurones. Photoreceptor cells possessed well developed outer segments. The basal processes of these cells formed (a) ribbon synapses with nerve cell processes and (b) areas of lateral neuropile with other photoreceptor cells containing gap junctions which may be sites of electrotonic conduction. Nerve cell processes were observed to form gap junctions with photoreceptor cells in areas of basal neuropile.

The interstitial cells contained large mitochondria and dilated smooth endoplasmic reticulum. The apical region of the cell was composed of (a) microvilli or (b) sheet like cytoplasm both of which bordered the lumen. These cells produce thin processes which were contained within the basal lamina and which penetrated the perivascular space. The processes were characterised by small vesicles which lay between the cell membrane and the basal lamina. Clear vesicles were found in cell processes near the basal lamina but the identity of these cells is uncertain.

Neurones were the least frequently found cell type and the cell bodies were positioned near the lumen of the organ. The nerve cell processes collect at the proximal region of the end vesicle before forming nerve bundles which combine to form the pineal tract which runs with the stalk The tract was observed to leave the stalk and to run between the posterior commissure and the sub commissural organ.

General Discussion

The seasonal constraints acting on temperate zone teleosts are severe and selection pressure to conform to the established breeding period must be considerable. It is not suprising therefore to find that the timing of spawning for species in mid to high latitude waters is usually precise. The reproductive cycle and breeding period of the powan are no exception, with timing apparently, strictly controlled. The spawning period of the powan has a precise starting point and lasts for little more than three weeks. The time interval between spawnings is therefore around eleven months. Scott (1979) questions that reproductive cycles of such long duration could be entirely endogenously timed. In the absence of any exogenous cues it would be even more remarkable that a female recruit to the breeding population, maturing in her third year, could through an endogenous rhythm alone, spawn in perfect synchrony with the rest of the adult population.

The environmental control experiments suggest that the natural progress of the reproductive cycle in Solea solea may be under the influence of an endogenous circannual rhythm. Moreover, the breeding population appears to attain a common synchrony in the initiation of vitellogenesis by a unified response to a photostimulatory photoperiod. There is also a suggestion that the reproductive cycle progresses at a set or basal rate, and the interval between cue and final maturation is fixed and can therefore be predicted. The rate appears to be determined by an endogenous rhythm which in the natural situation would be entrained by the photoperiod and influenced by a natural cycle of temperature. This is in general agreement with the hypothesis of a basal rate of ovarian maturation (Scott, 1963) but differs from it by involving an endogenous rhythm.

Recent evidence suggests that it is the long daylengths of spring and summer that initiate gonad recrudesence in salmonids (Bromage, Whitehead and Breton, 1982), in contrast to the widely held view that it is the

decreasing daylengths of late summer and autumn. It was found that in a winter (January) spawning strain of Salmo gairdneri vitellogenin occurred in the blood during May and increased thereafter until spawning. Although vitellogenin has not been measured in the blood of the powan, vitellogenesis does begin during the summer, which suggests that recrudesence is in progress from an early stage, possibly spring. Confirmation of this is required by a histological study of the ovary.

After the physiological demands of reproduction have been met, individuals are faced with the task of restoring their overall body condition. Although the condition factor is a relatively crude indicator of physiological well being and is open to misinterpretation, it does provide an index of general condition which is cyclical and regularly repeated. It is a feature of this cycle, that in both Coregonus lavaretus and Solea solea there is a significant increase in condition at the time both species begin exogenous vitellogenesis. The evidence from Solea solea suggests that the populations respond to a photostimulatory cue after the rapid rise in condition. Furthermore the observation that it is possible to acheive an unnatural advance in spawning time by exposing the fish to an early cue suggests that some individuals are primed to respond in advance of the natural cue.

The advantages of a common synchrony in reproductive activity to both the individual and the breeding population are obvious. It is possible however, that there may be additional benefits in having a synchronising cue after the population recover condition. It would allow for most of the fish to reach a certain (possibly threshold) condition prior to starting the most physiologically demanding phase of the reproductive cycle, and accomodate individual variation in recovery rate. Moreover, it would synchronise the reproductive cycle of three separate groups within the breeding population: new recruits who have no need to recover from a previous spawning, post spawning fish, and adults out of phase with the

annual cycle.

Such a system might provide a population regulating mechanism as for the great majority of animals the critical resource which ultimately determines population density is food. If there is a critical threshold of condition linked to a photosensitive phase in the reproductive cycle, fish which do not make condition in time will have their cycle out of synchrony with the main breeding population. Although they would continue to reach sexual maturity the chance of spawning successfully would be remote. This would explain the random occurrence of mature females months out of phase with the main population. If there were too many fish in the population,and the food resources stretched,fewer individuals would make the condition threshold in time and would therefore be unable to respond to the cue. Maturing out of synchrony with the main breeding population would be likely to prevent a contribution to the next year class. A food supply which was not critical would enable the majority of individuals to make the threshold and thereby maximise larval production.

Solea solea females will ovulate and spawn irrespective of the photoperiod provided that the temperature is equal to or above 8°C. Ova incubated below this temperature suffer high mortality (O'Connell, unpublished). It seems therefore that spawning was delayed until the sea temperature had stabilised around 8°C. Ova hatched 8 to 9 days after fertilisation at this temperature. The ova of Coregonus lavaretus were susceptible to temperatures equal to or above 9°C (Zuromska, 1982) and hatching occurred between 40 to 80 days after fertilisation in the laboratory (6 to 9°C) but took longer in Loch Lomond where the range was 3 to 6°C (Maitland, 1967 b).

Both Solea solea and Coregonus lavaretus are temperate zone species producing pelagic larvae in the spring. The reproductive strategies are different yet the objectives are the same. One species could ensure directly that hatching occurred at the optimal time but the other had to predict the

the temperature over three months in advance. Moreover, there are further constraints on hatching time as whitefish larvae have very specific food requirements and are highly sensitive to lack of food (Flüchter, 1979). Hatching therefore, must occur in synchrony with the availability of food (for post-yolk sac larvae), or high mortalities will result (Zuromska, 1982).

Temperatures in Loch Lomond at the spawning time differ between regions; the rate of change is also very slow and unlikely to provide a suitable stimulus. The fish must therefore find some feature in the environment which can provide them with an accurate means of measuring time, and also act as a synchronising cue for the breeding population to begin spawning. Solar photoperiod changes minimally at this time and does not account for the spread in spawning times. The correlation between the beginning of the spawning period and the full moon requires to be verified experimentally, yet seems to fulfil the criteria. To be effective the fish would have to monitor the cycle of full moons and be entrained to their period (Scott, 1979). The appearance of Coregonus lavaretus in the surface layers during the night may be a behavioural adaptation to minimise the effect of attenuation and increase the strength of the light signal.

The occasional ovulated female was found in samples up to six months out of phase with the breeding population, and these odd females also occur in European whitefish Coregonus lavaretus and vendace Coregonus albula populations (Zuromska, 1982). If the fish are entrained to a lunar rhythm, it is possible that out of phase females would ovulate spontaneously. Spawning in association with lunar phases is known in other teleosts (Walker, 1949; Lowe-McConnell, 1979). An effect of lunar photoperiodicity is not unknown in salmonids; in salmon the thyroxine surge associated with smoltification is precisely timed to coincide with the new moon phase of the lunar cycle, and smolt migration to the sea occurs at full moon (Grau et al, 1982).

Spawning is normally regarded as the successful completion of the

the reproductive cycle in teleosts, which is not strictly true. The hatching date is a more realistic termination point, for until this stage is reached the cycle cannot be termed successful. In both species it is temperature which ultimately determines hatching time, and therefore completion of the cycle.

The reproductive cycle of Solea solea appears to be self sustaining and can be completed successfully (in captivity) in the absence of a natural photoperiod. Similar findings have been reported for other temperate zone teleosts. The minnow Phoxinus phoxinus (Scott, 1979), and the rainbow trout Salmo gairdneri = Salmo irideus [Gibbons] (Bieniarz, 1973) both reached sexual maturity in the absence of a natural photoperiod. In both minnows and sole sexual maturation occurred out of synchrony with the natural breeding period when the environmental timing cues were removed. For Solea solea it is the absence of a photostimulatory cue, present during a short period, which eliminates spawning synchrony in the breeding population. What happens to the temperature and photoperiod after the cue appears to be irrelevant to the natural progress of the reproductive cycle (excepting temperature shocks). This suggests that the function of the photostimulatory cue is to synchronise the initiation of a physiological phase within the breeding population. For Solea solea this is probably exogenous vitellogenesis.

It would be remarkable for a fish to respond to a photostimulatory cue if it did not already monitor the daily photoperiod. The effect of photoperiod on the entrainment of endogenous circadian rhythms in vertebrates is well established (Bunning, 1973; Saunders, 1977) and in teleosts (de Vlaming & Olcese, 1981; Kavaliers & Ross, 1980). Moreover, hormones associated with reproduction and the hypothalamo - pituitary - gonad axis are known to follow a circadian rhythm in teleosts (Crim, 1982). An endogenous rhythm therefore probably ensures the temporal coordination of physiological processes connected with reproduction in Solea solea, but

it does not appear to directly control the synchronisation of the cycle.

Several models have been proposed to explain the photoperiodic response of the neuroendocrine system. In the external coincidence model it is assumed that light acts as a signal to synchronise the endogenous, circadian rhythm of photosensitivity, and that light acting as a stimulus falls in a period of photosensitivity and thus induces a response (Pittendrigh and Minis, 1964). In the internal coincidence model light serves as a signal and not as a stimulus. The model assumes that there are at least two oscillators in which one is phased by dawn and the other by dusk. When the phases of the two internal oscillators are in optimal configuration, the maximal response occurs (Saunders, 1977). The individual merit of the models is not discussed here, but they provide a basis for understanding the response to photoperiod in Solea solea.

If teleosts possess endogenous rhythms which require to be entrained by the photoperiod, they will need a transducing system. The evidence supporting a photoreceptive role for the pineal organ is strong. Moreover, electrophysiological studies have demonstrated that the salmonid pineal has a dark adapted threshold of 3×10^{-5} lumens/m^2 (Morita, 1975). Allowing 0.5 log units for attenuation through the skin and skull (Falcon & Meissl, 1981), the sensitivity of the organ is well within the range of light intensities experienced during the twilight period. This has led to the speculation that the teleost pineal may be involved in the measurement of daylength (Falcon & Meissl, 1981; and others). However, direct evidence linking the teleost pineal to circadian organisation is limited (Kavaliers, 1981). This is probably due to a limited number of workers in the field rather than lack of supportive evidence. There is growing evidence indicating that the teleost pineal acts as a mediator between seasonal photoperiod and many physiological systems including reproduction (deVlaming, 1982). Moreover, the evidence reviewed suggests that the pineal may represent a link between the photoperiod and the synchronisation of

circadian rhythms.

The evidence suggests that the twilight migrations of Coregonus lavaretus are precisely timed and related to low light intensities. Moreover, these migrations into the surface water persist when the population is known not to be feeding on plankton. In common with other salmonids the coregonine pineal organ shows morphological signs of a photoreceptive role but this requires to be verified by electro-physiological experiment. It is possible therefore that the twilight migrations to the surface layers are an adaptation to minimise the effect of attenuation and to increase the strength and accuracy of the photo-period time signal.

The mammalian pineal organ is not directly photoreceptive and therefore its function is possibly of limited relevance to teleosts. It has been clearly shown however that the mammalian pineal influences various endocrine systems besides those regulating reproduction (Hoffman, 1981; Lincoln & Short, 1980). This is in general agreement with a recent review of possible pineal function in teleosts (de Vlaming, 1982). However, in teleosts the evidence supporting a role of the pineal in the regulation of reproduction is conflicting. Some workers report no effect on seasonal reproduction (Day & Taylor, 1983) while others support an inhibitory and stimulatory role (Urasaki, 1976). In many of these experiments no allowance has been made for seasonal variation in responsiveness to photoperiod or the effect of an endogenous circannual rhythm.

Melatonin is regarded as having an inhibitory role in the regulation of gametogenesis and is produced maximally during the dark phase of the photoperiod. The inhibitory role of the pineal should therefore be maximally active during the winter months yet both Solea solea and Coregonus lavaretus mature their gonads during this period. Furthermore, rainbow trout Salmo gairdneri = Salmo irideus [Gibbons] were reared to sexual maturity in continuous light and continuous darkness from fertilisation

(Bieniarz, 1973). The pineal organ appears to have limited importance in the regulation of reproductive cycles, but it may function by mediating photoperiod information to the hypothalamo - pituitary complex where control of reproduction lies.

If the pineal is linked to circadian and circannual organisation within the animal, its removal would possibly destroy temporal coordination and cause the expression of 'free-running' periods. The effect would be the elimination of reproductive synchrony within breeding populations rather than a breakdown in the regulation of the reproductive cycle. In the natural environment of the animal the pineal organ would therefore be important to the timing of the reproductive cycle.

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Addendum :

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The experiments on sole (Solea solea) were conducted at the White Fish Authority's Marine Farming Unit, Hunterston and my thanks are due to the staff there especially Mr J. Arthur for help with the husbandry.

Plate 1

An aerial photograph of the survey area. Possible shallow water spawning sites are shown by a white arrow. Details of this area can be found in figure 1.

The scale line corresponds to a length of 200 metres.

Plate 2

Sagittal section through the fore-brain, 5µm, Masson's Trichrome.
This section shows the position of the pineal organ in the adult powan.

ds - dorsal sac
hc - habenular commissure
mes - mesencephalon
nh - nuleus habenulae
p - paraphysis
pc - posterior commissure
pev - pineal end vesicle
ps - pineal sinus
ps - pineal stalk
sco - sub-commissural organ
tel - telencephalon
vt - velum transversum

The scale line corresponds to a length of 150 µm.

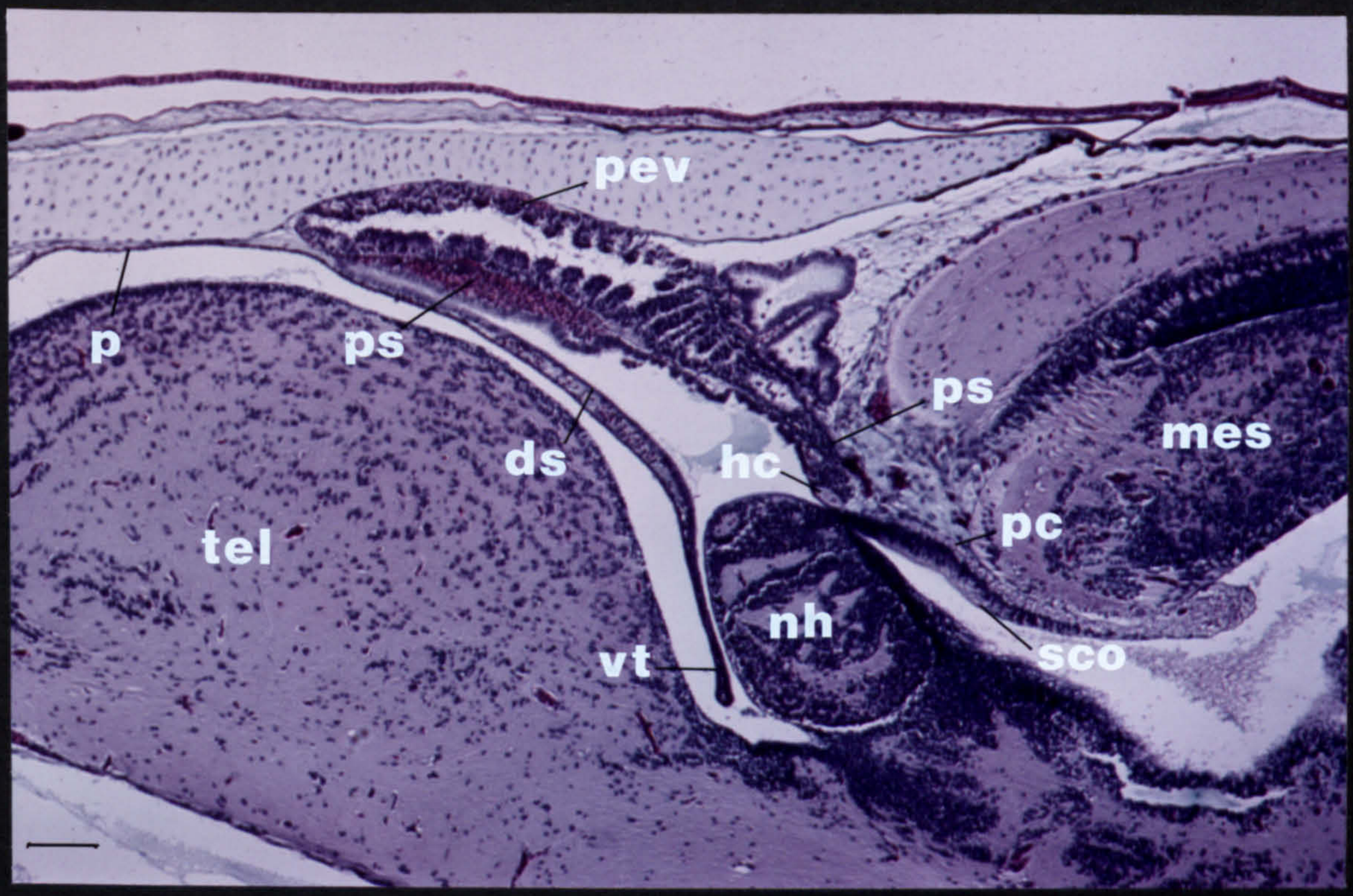
Plate 3

Sagittal section through the ventral wall of the pineal organ, 5 µm, Masson's Trichrome.
This section shows the general relationship of the pineal epithelium to the lumen and blood vessels.

bp - basal process of photoreceptor cell
ctc - connective tissue capsule (collagen)
e - erythrocyte
mm - meningeal melanophores
n - neuropile
os - outer segment
pc - photoreceptor cell nucleus
pvs - peri-vascular space

The scale line corresponds to a length of 50 µm.

2



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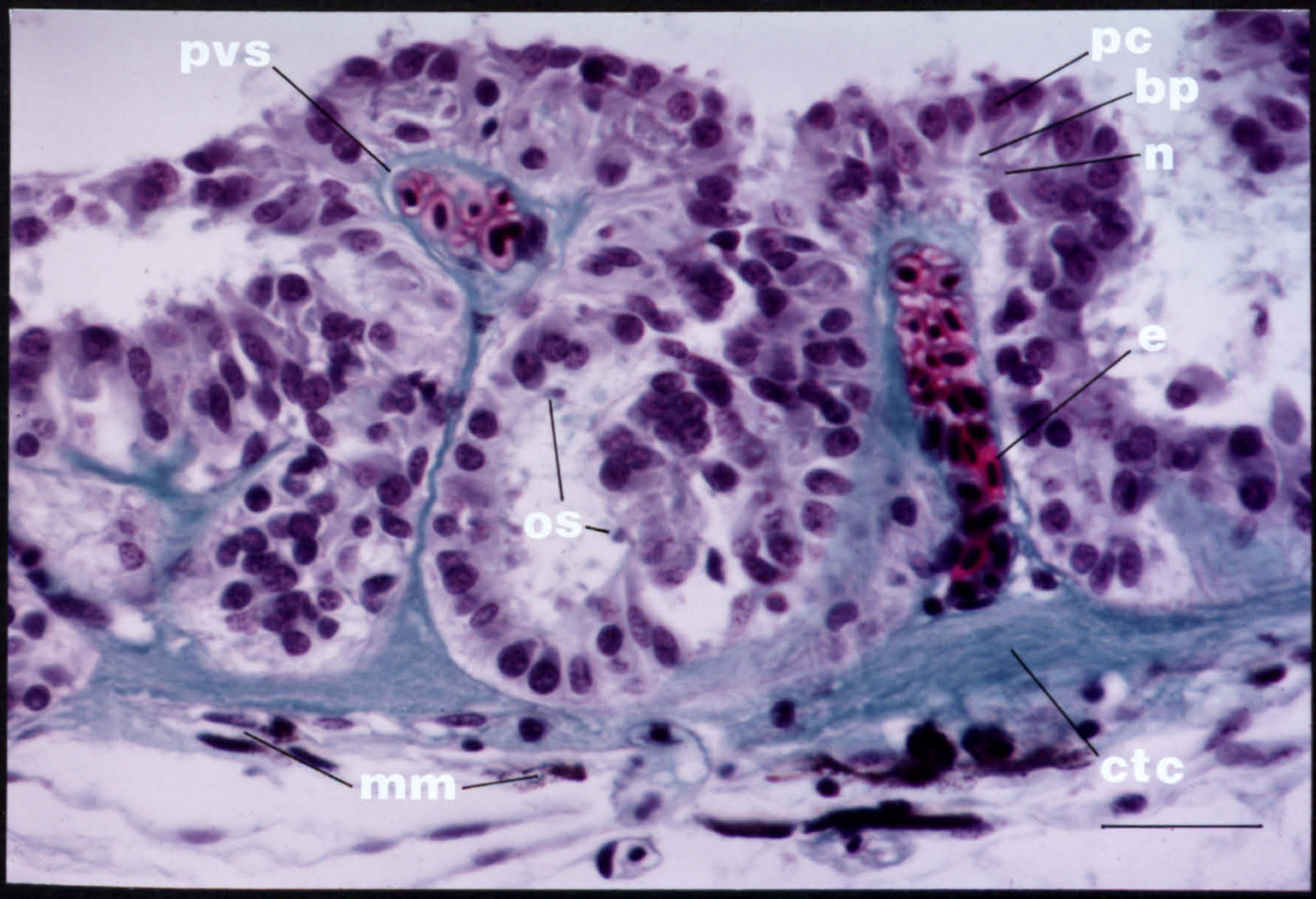


Plate 4

Section through the pineal stalk and habenular commissure, 5 μm, Masson's Trichrome.
This section shows the relationship of the pineal nerve tract to the stalk, near to the point at which the lumen of the stalk opens to the third ventricle.

ds - dorsal sac
hc - habenular commissure
nt - nerve tract
ps - pineal stalk
sco - sub-commissural organ

The scale line corresponds to a length of 100 μm.

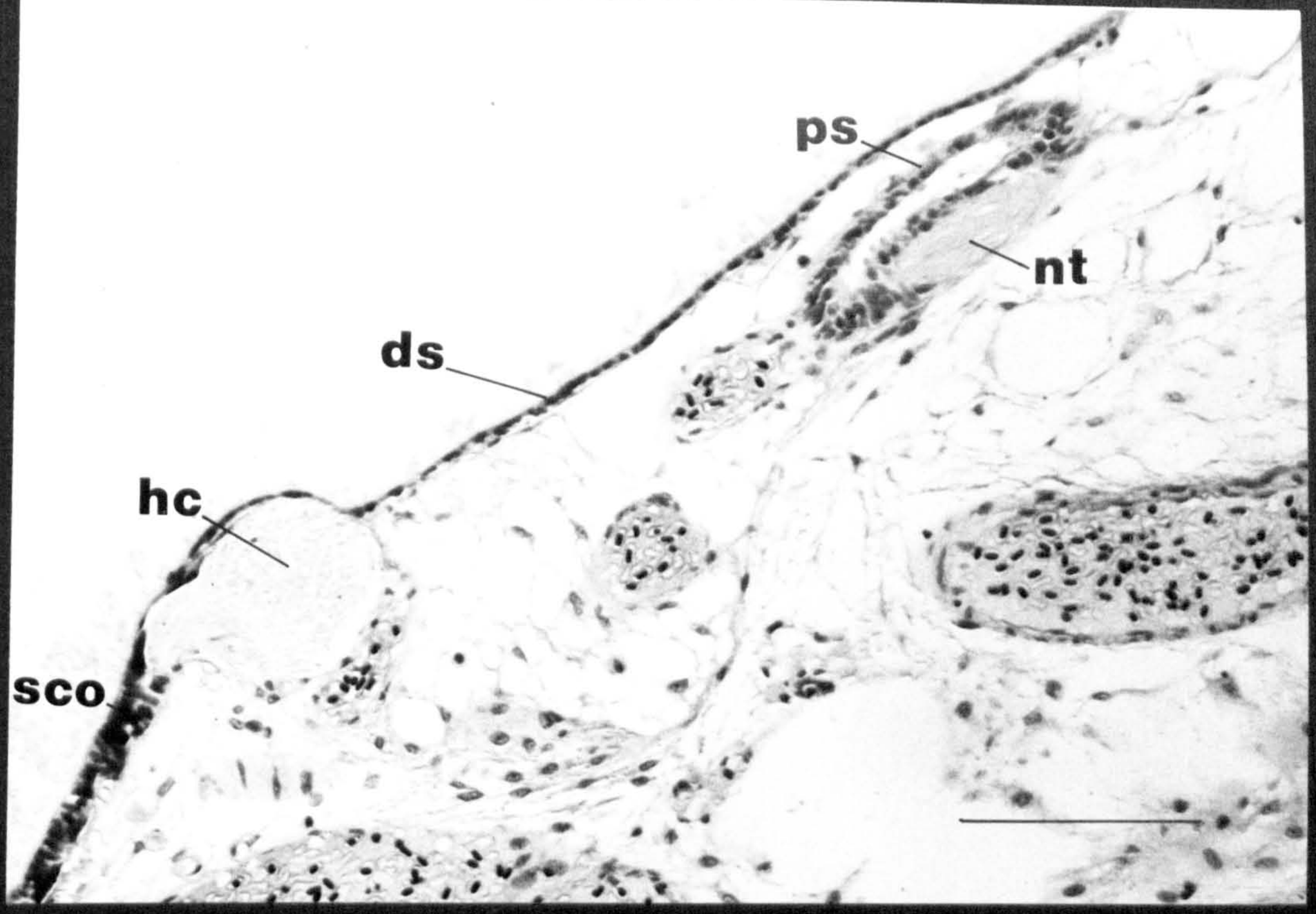
Plate 5

Section through the epiphyseal arch, 5 μm, Masson's Trichrome.
The sub-commissural organ and the habenular commissure, together, comprise the epiphyseal arch.
This section shows the pineal nerve tract at the point where it starts to course between the sub-commissural organ and the posterior commissure.

pc - posterior commissure
pt - pineal nerve tract
sco - sub-commissural organ

The scale line corresponds to a length of 50 μm.

4



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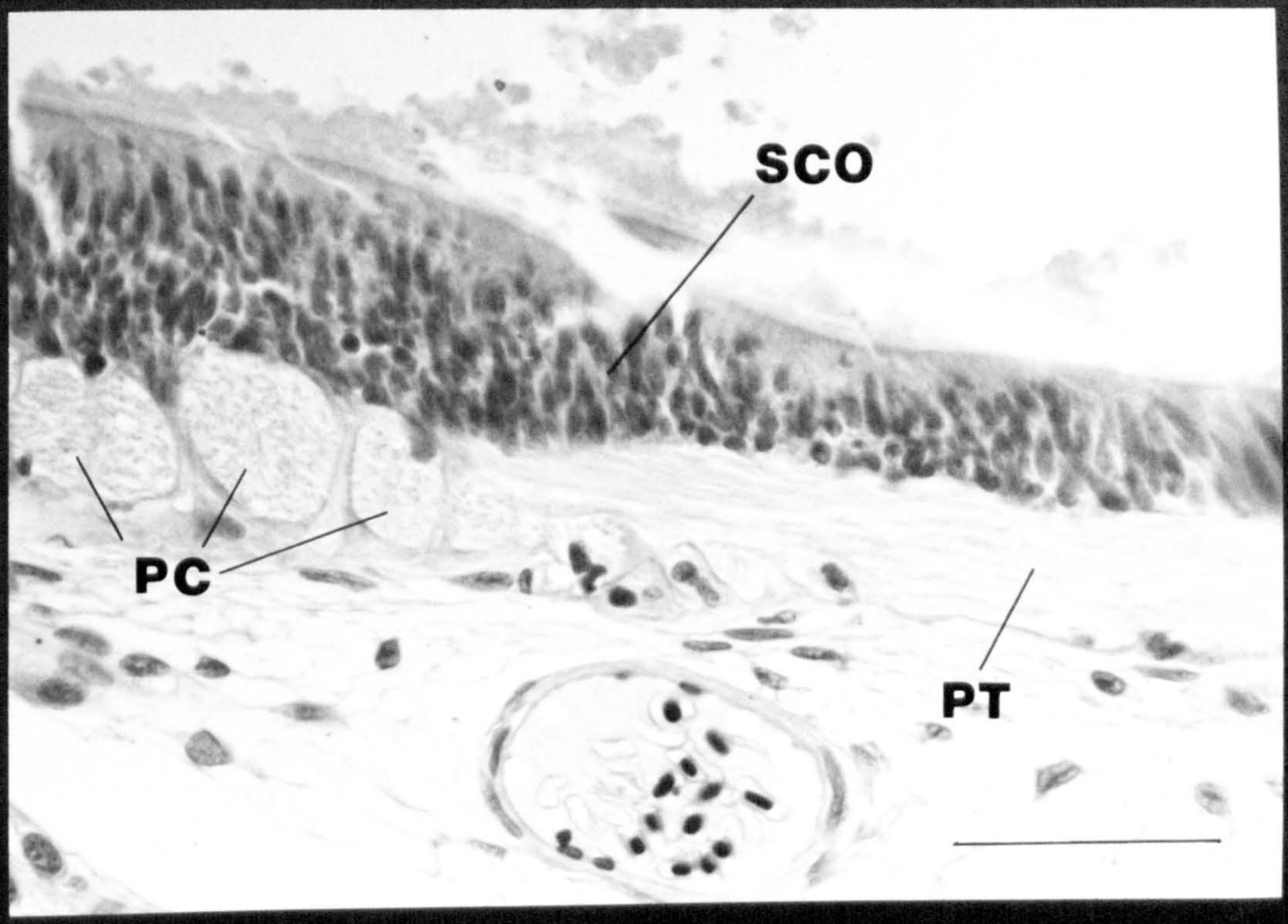


Plate 6

A section through the pineal epithelium at the interface with the collagen matrix, 5 μm, Masson's Trichrome. This section shows the basal processes of interstitial cells which terminate at the basal lamina; the apical process is more difficult to identify, but is usually narrower than the apical process of a photoreceptor cell.

bv - blood vessel

ic - interstitial cell

pvs - peri-vascular space

long arrows - interstitial cell basal process

short arrow - " " apical process

The scale line corresponds to a length of 20 μm.

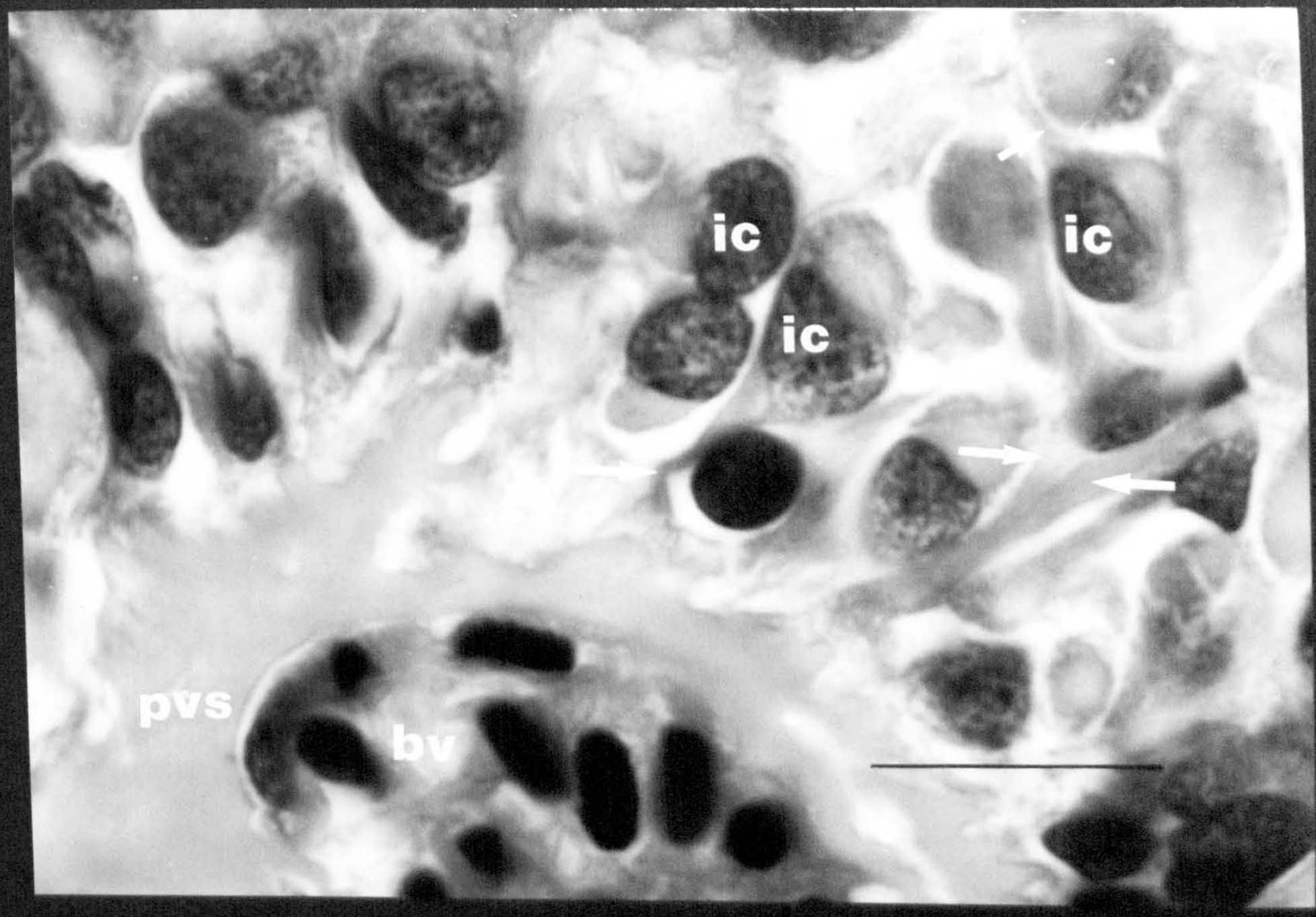
Plate 7

Transverse section through the proximal region of the pineal end-vesicle, 5 μm, Masson's Trichrome. This section shows the extensive nerve tract which occur in the basal epithelium.

nt - nerve tract

The scale line corresponds to a length of 50 μm.

6



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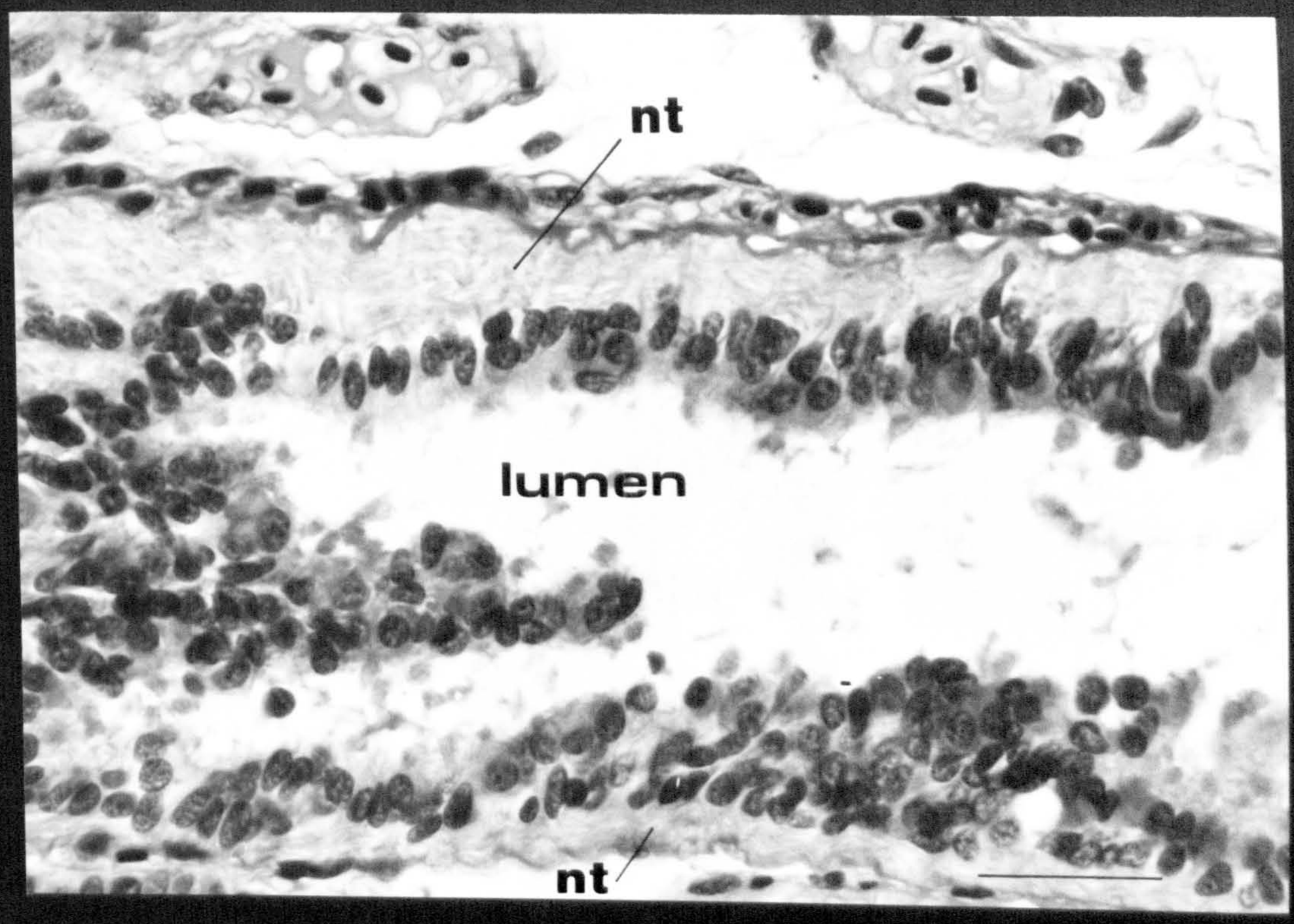

Plate 8

Sagittal section through the dorsal region of the diencephalon, 5μ, Masson's Trichrome.
This section shows the pineal organ in the powan embryo (55 days, post fertilisation), and that the lumen is continuous with the third ventricle.

ds - dorsal sac

hc - habenular commissure

p - pineal end-vesicle and stalk

pl - pineal lumen

pc - posterior commissure

sco - sub-commissural organ

t - telencephalon

vt - velum transversum

The scale line corresponds to a length of 25 μ.

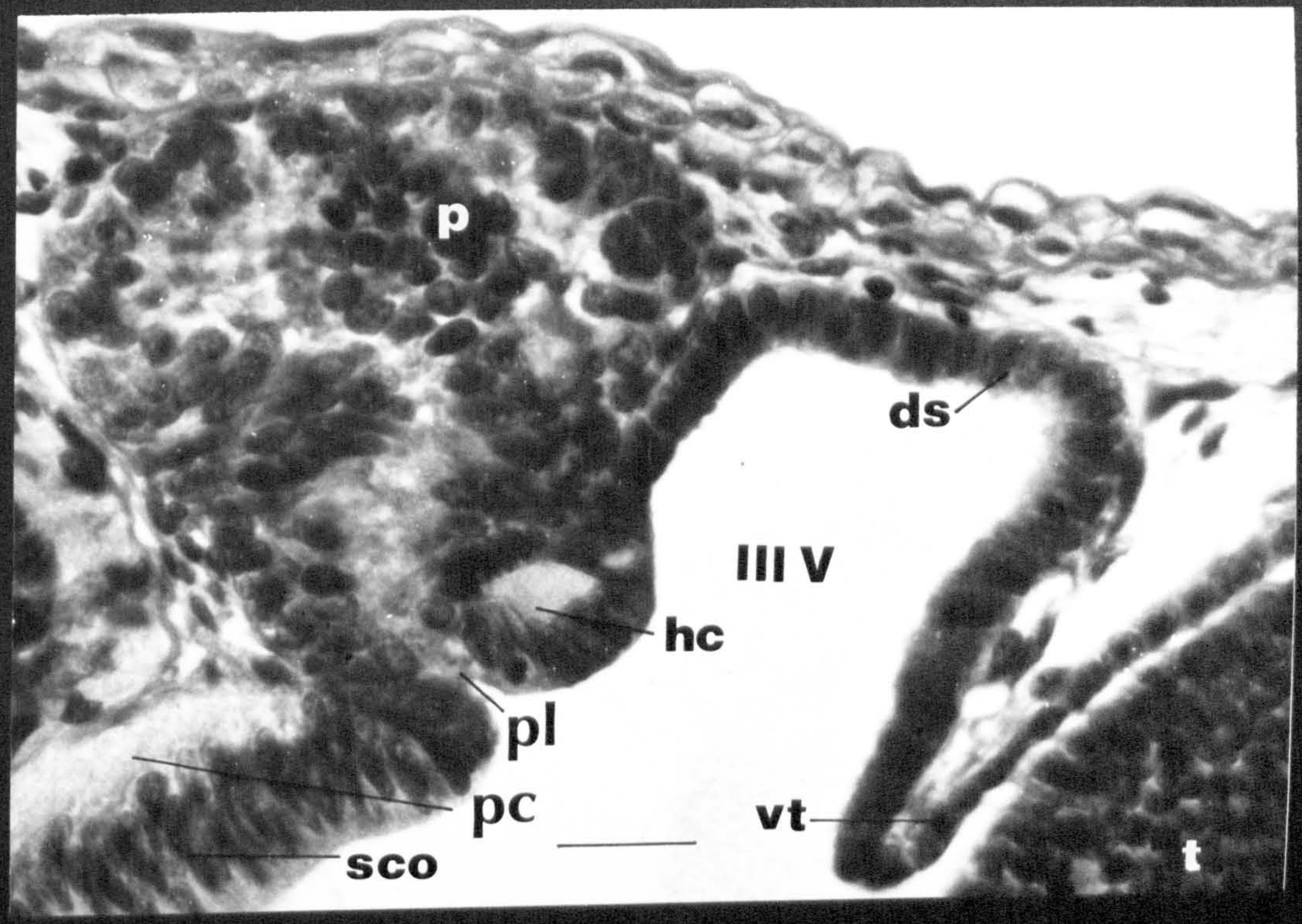
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Plate 9

Transverse section through the roof of the diencephalon, 5 μm, Masson's Trichrome.
This section shows the relationship between the pineal stalk, habenular commissure and the parapineal organ.

HC - habenular commissure
M - mesencephalon
PL - pineal lumen
PP - parapineal organ
PPN - parapineal nerve tract
PS - pineal stalk
SCO - sub-commissural organ

The scale line corresponds to a length of 25 μm.

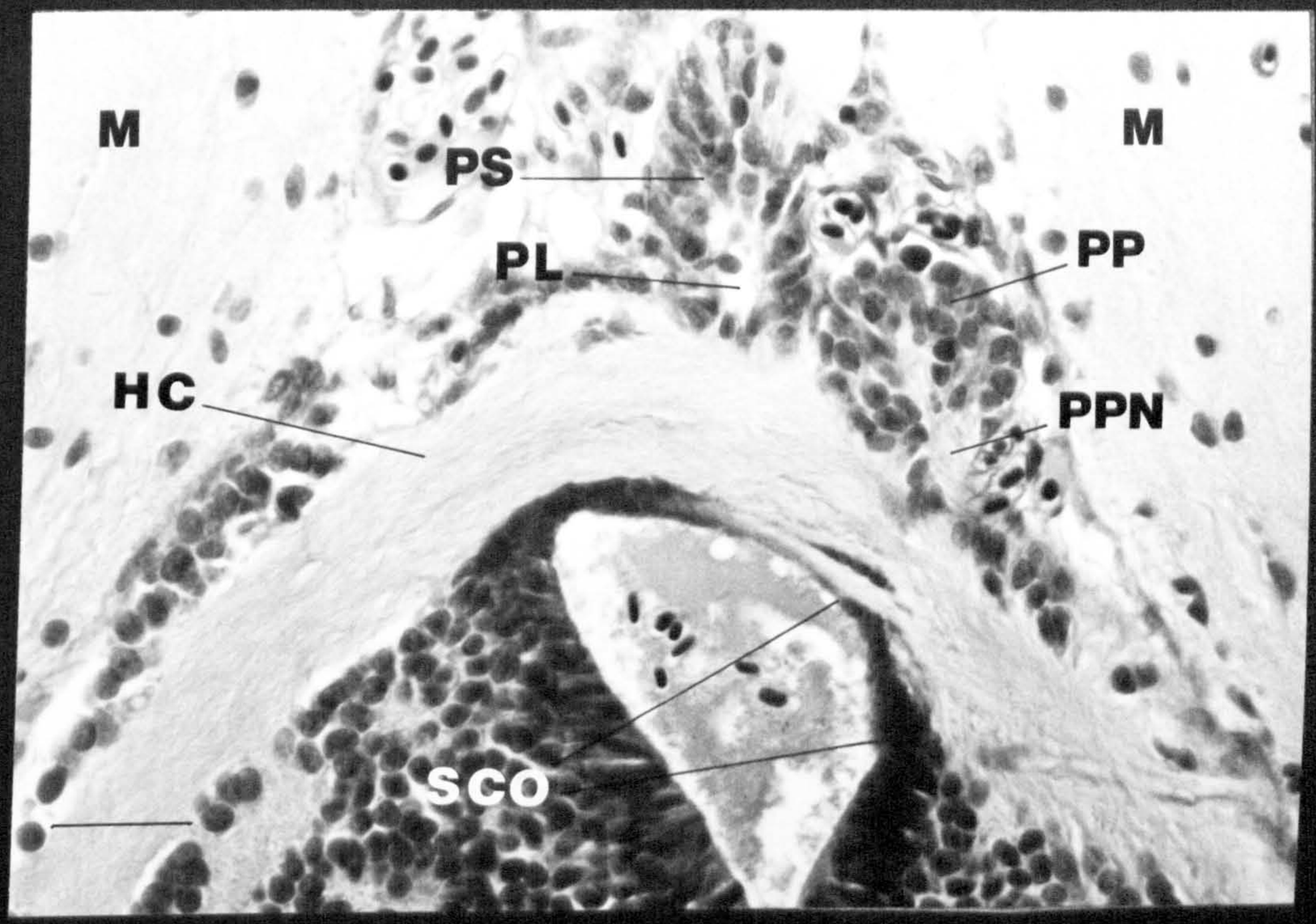
Plate 10

Transverse section through the roof of the diencephalon, 5 μm, Masson's Trichrome.
This section shows the pineal stalk joining the sub-commissural organ, and that the pineal lumen is continuous with the cerebrospinal fluid of the third ventricle. The section is taken from a 22 day old fry.

CSF - cerebrospinal fluid
PL - pineal lumen
PP - parapineal organ
PS - pineal stalk
M - mesencephalon
SCO - sub-commissural organ

The scale line corresponds to a length of 25 μm.

9



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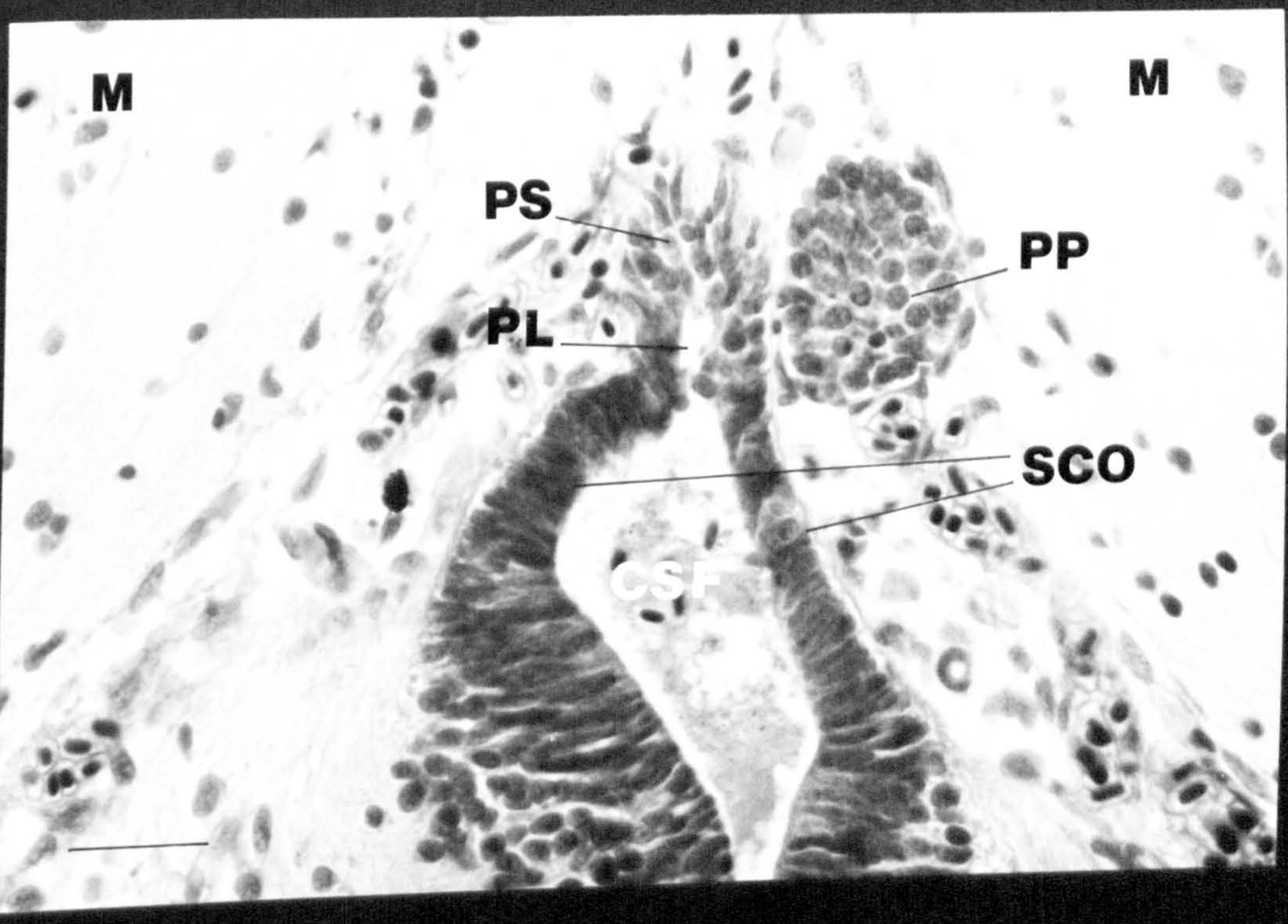


Plate 11

The outer segment and cilium (9+0) of a photoreceptor cell in a powan embryo (55 days, post fertilisation).

OS - outer segment
C - 9+0 cilium

The scale line corresponds to a length of 1 μm.

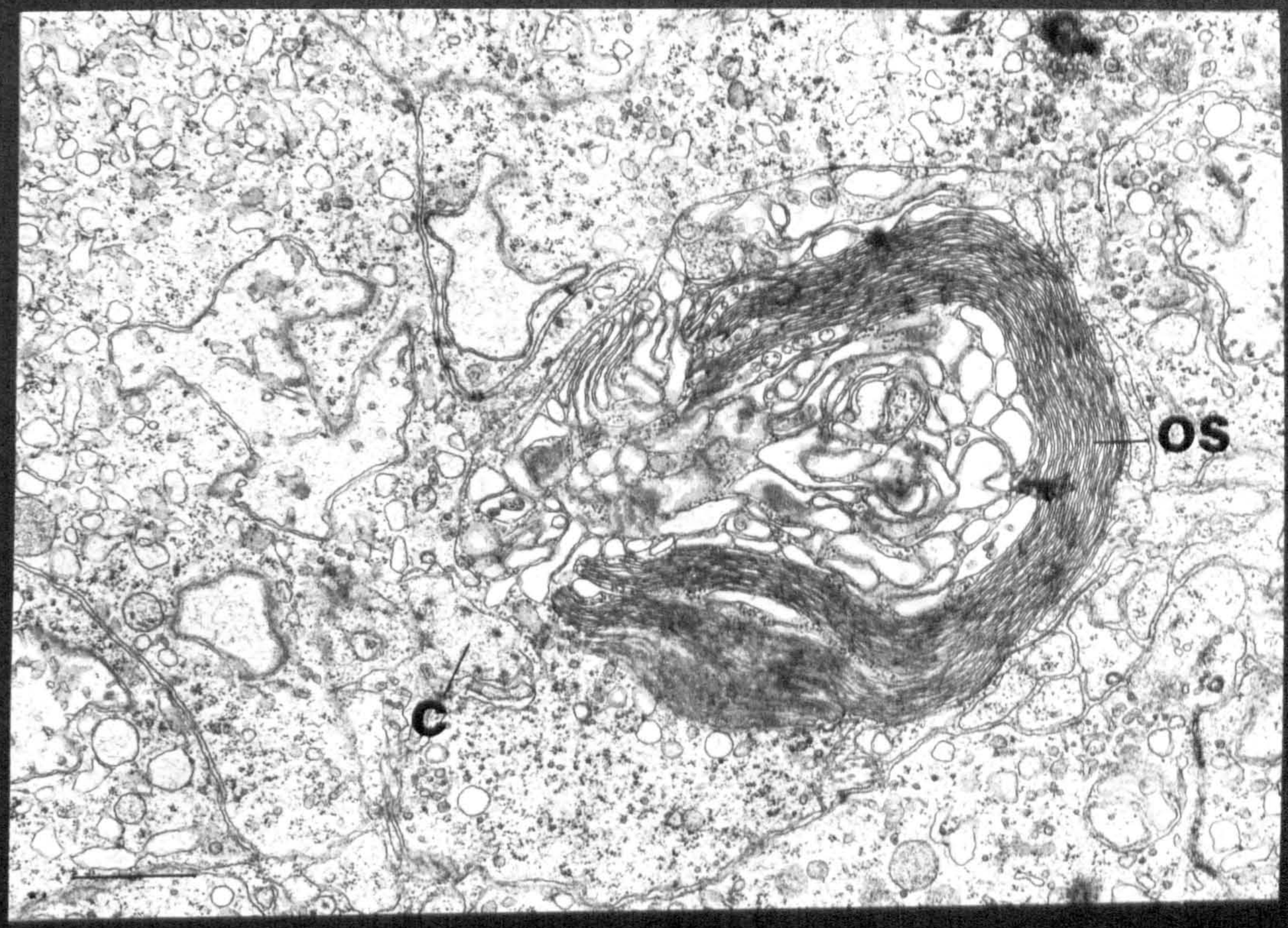
Plate 12

The arrangement of photoreceptor and interstitial cell apical processes bordering the lumen.

IS - inner segment, photoreceptor cell
mi - mitochondrion, interstitial cell
mp - mitochondrion, photoreceptor cell
mv - microvilli, interstitial cell
ser - smooth endoplasmic reticulum

The scale line corresponds to a length of 4 μm.

11



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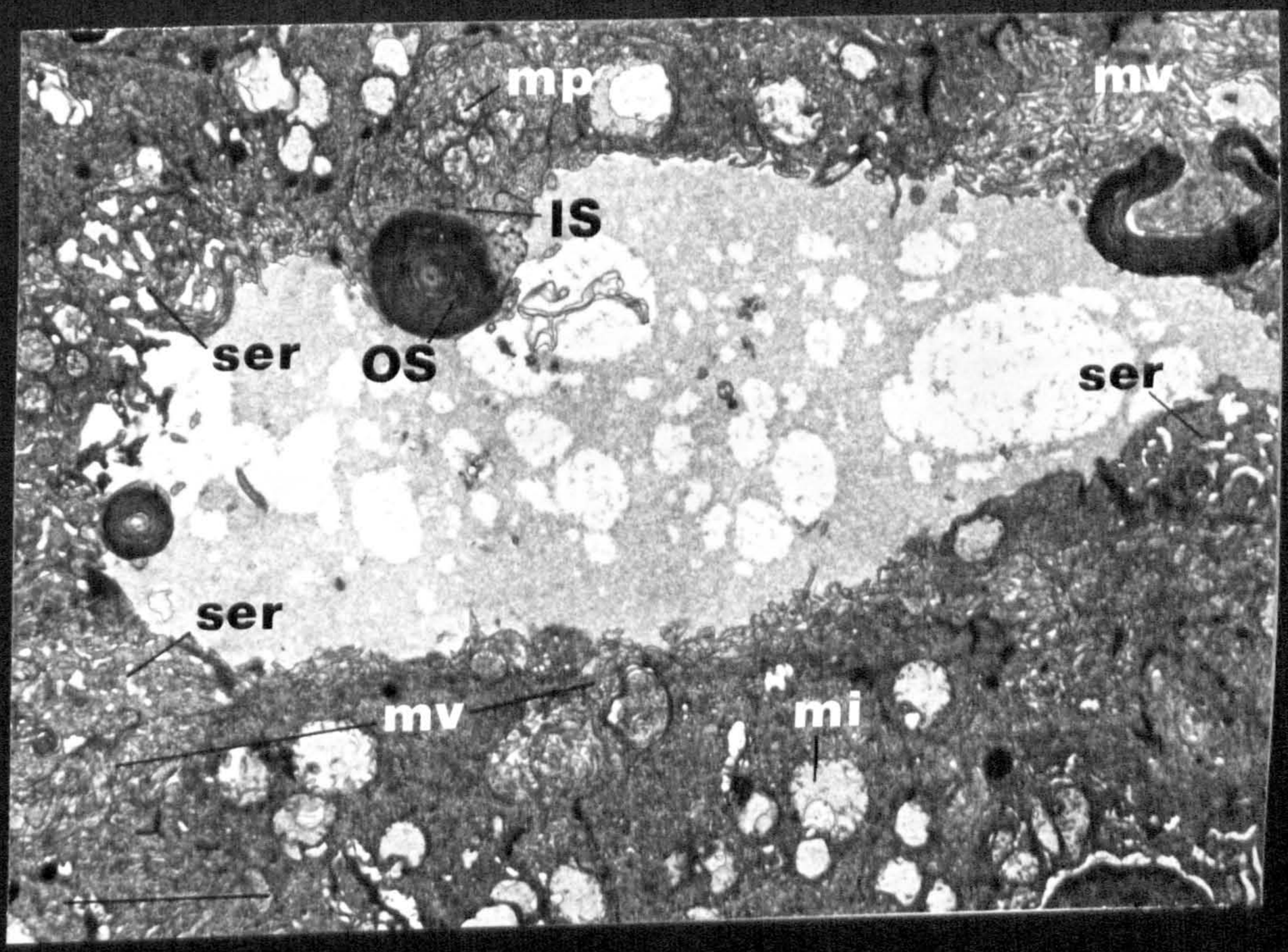


Plate 13

A photoreceptor cell outer and inner segment, surrounded by the microvilli and apical processes of interstitial cells.

ic - interstitial cell

is - inner segment

mv - microvilli

n - neck region

os - outer segment

za - zonula adherens

small arrows - tubular structures which lie along the outer segment

thick arrow - lamellae of the outer segment

The scale line corresponds to a length of 1 μm.

Plate 14

The inner and outer segments of photoreceptor cells, showing the extensive smooth endoplasmic reticulum and microfilament bundles. The tubule formation within the outer segment and the dilated cisternae of smooth endoplasmic reticulum may be associated with the breakdown of the outer segment.

c - supporting cilium for the outer segment

IS - inner segment

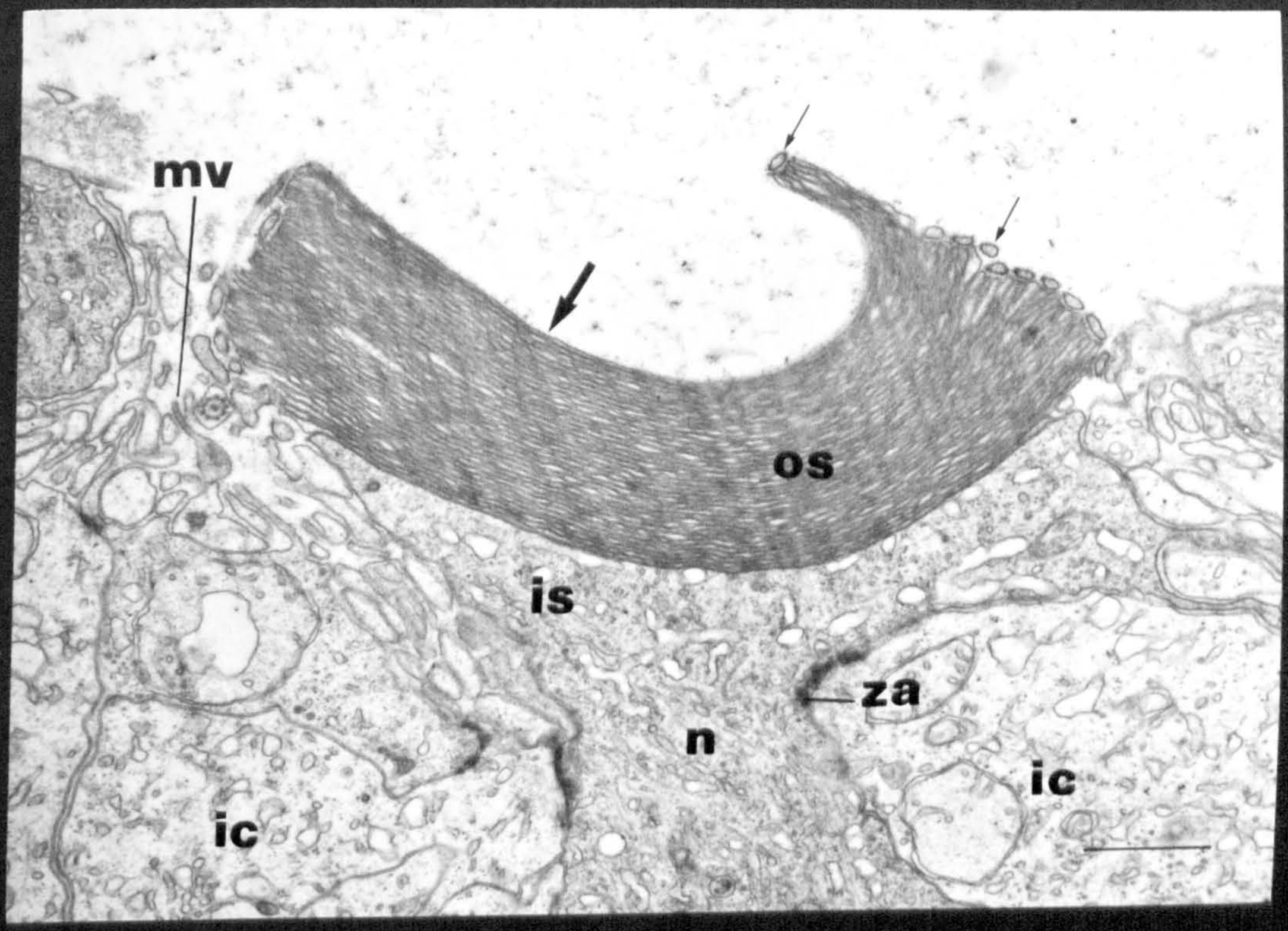
mf - microfilament bundle

ser - smooth endoplasmic reticulum

arrows - tubular complex in outer segment

The scale line corresponds to a length of 1 μm.

13



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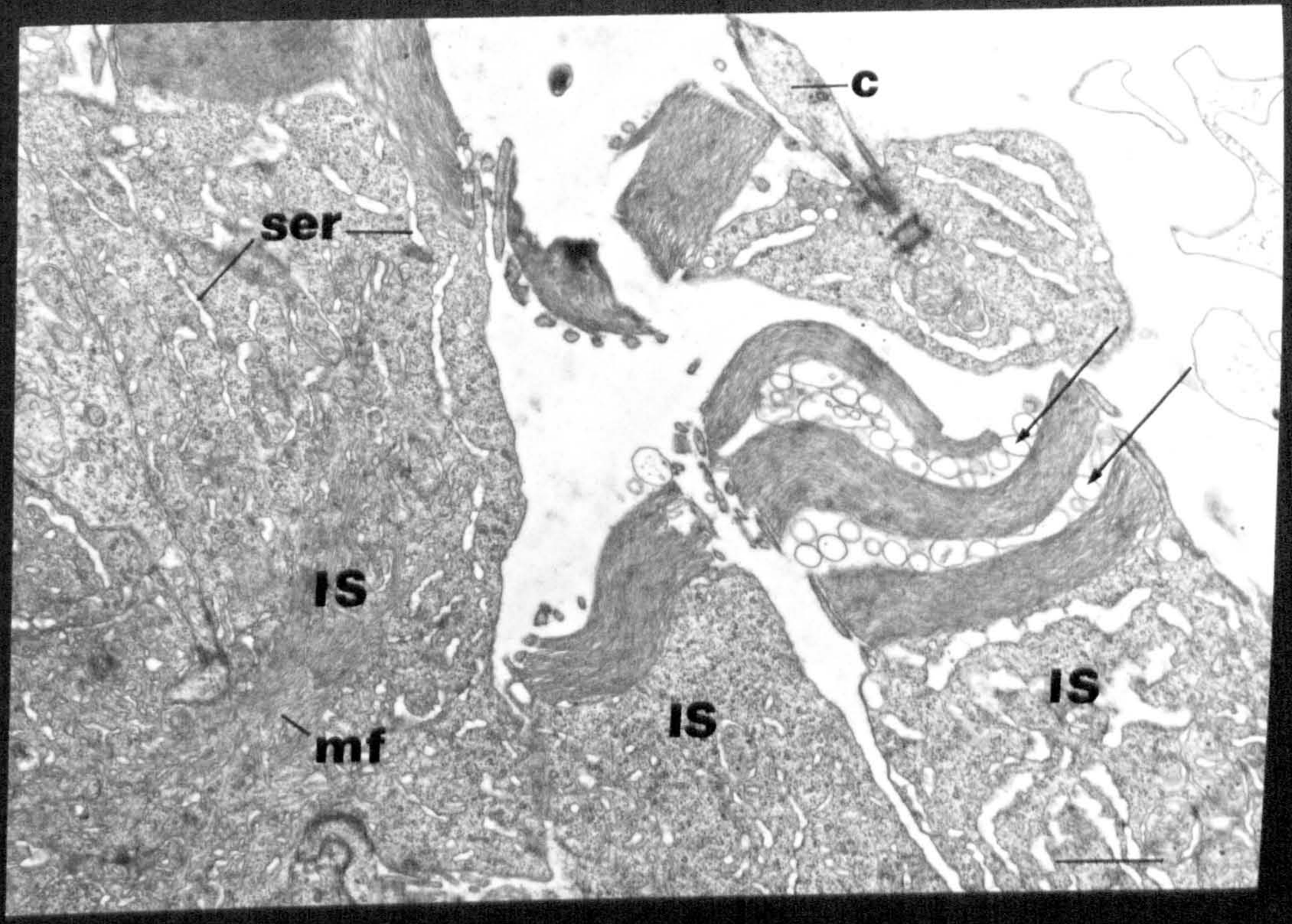


Plate 15

Photoreceptor outer segment of the 'horse-shoe' type and an inner segment containing granular bodies. The apical region of the interstitial cell is characterised by extensive microvilli.

g - Golgi apparatus
gb - granular body
m - mitochondrion
mv - microvilli
pc - photoreceptor cell

The scale line corresponds to a length of 2 μm.

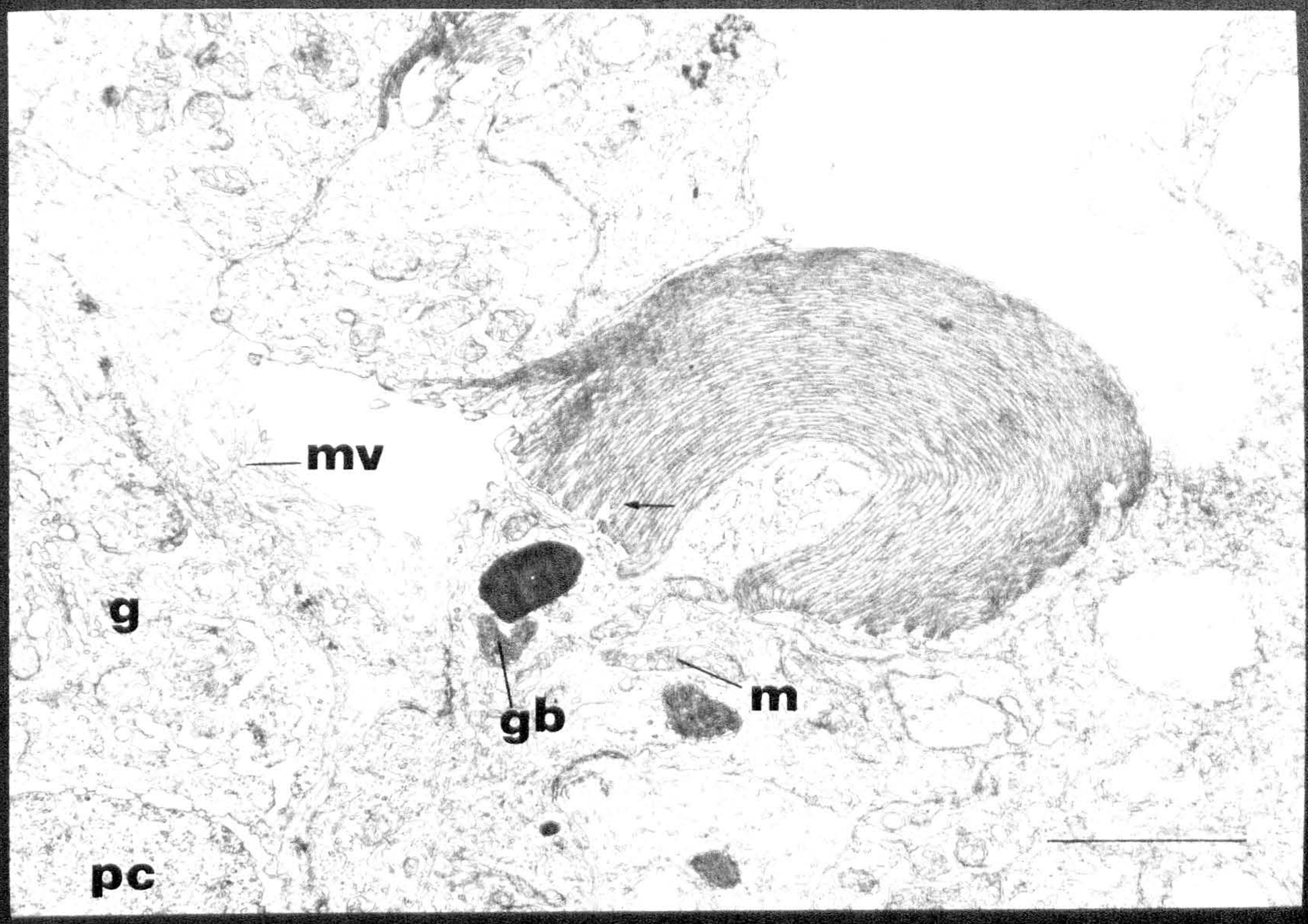
Plate 16

The main body of a photoreceptor cell, showing the characteristic smooth endoplasmic reticulum, and Golgi apparatus. The adjacent interstitial cells contained large mitochondria. The disintegrated outer segment of a photoreceptor cell appears to be associated with the apical process of an interstitial cell.

dcv - dense cored vesicles
dos - disintegrated outer segment
ic - interstitial cell
m - mitochondrion, interstitial cell
pc - photoreceptor cell
pnc - peri-nuclear cisternae
ser - smooth endoplasmic reticulum
arrows - Golgi apparatus

The scale line corresponds to a length of 2 μm.

15



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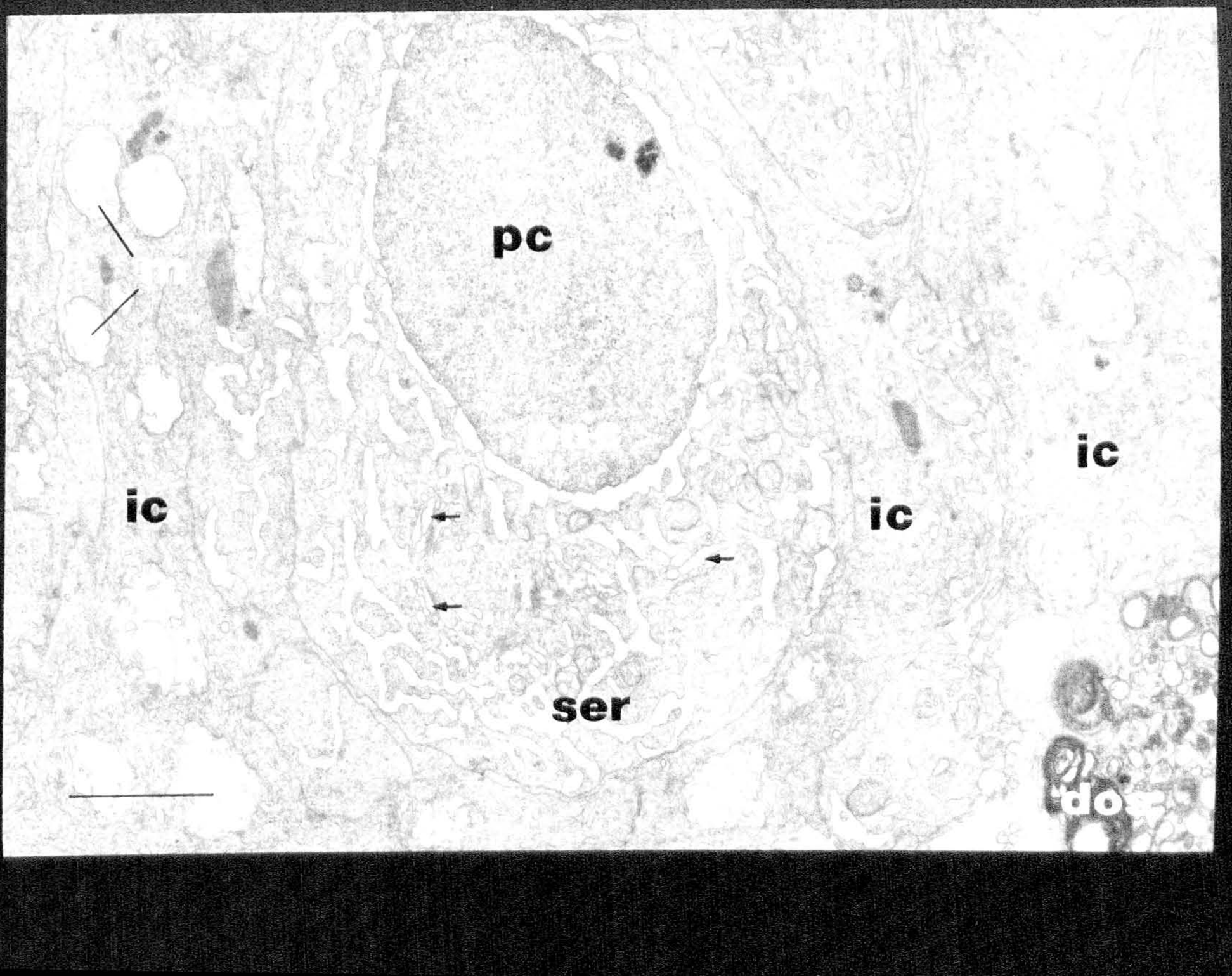


Plate 17

The apical processes of photoreceptor cells, showing collections of granular bodies. These were often found in association with the sheet-like,apical process of interstitial cells. Although not shown, micro-villi still occur at regular intervals within the areas of 'sheet' formation.

g - golgi body
gb - granular bodies
IC - interstitial cell
PC - photoreceptor cell (main body)

The scale line corresponds to a length of 1μm.

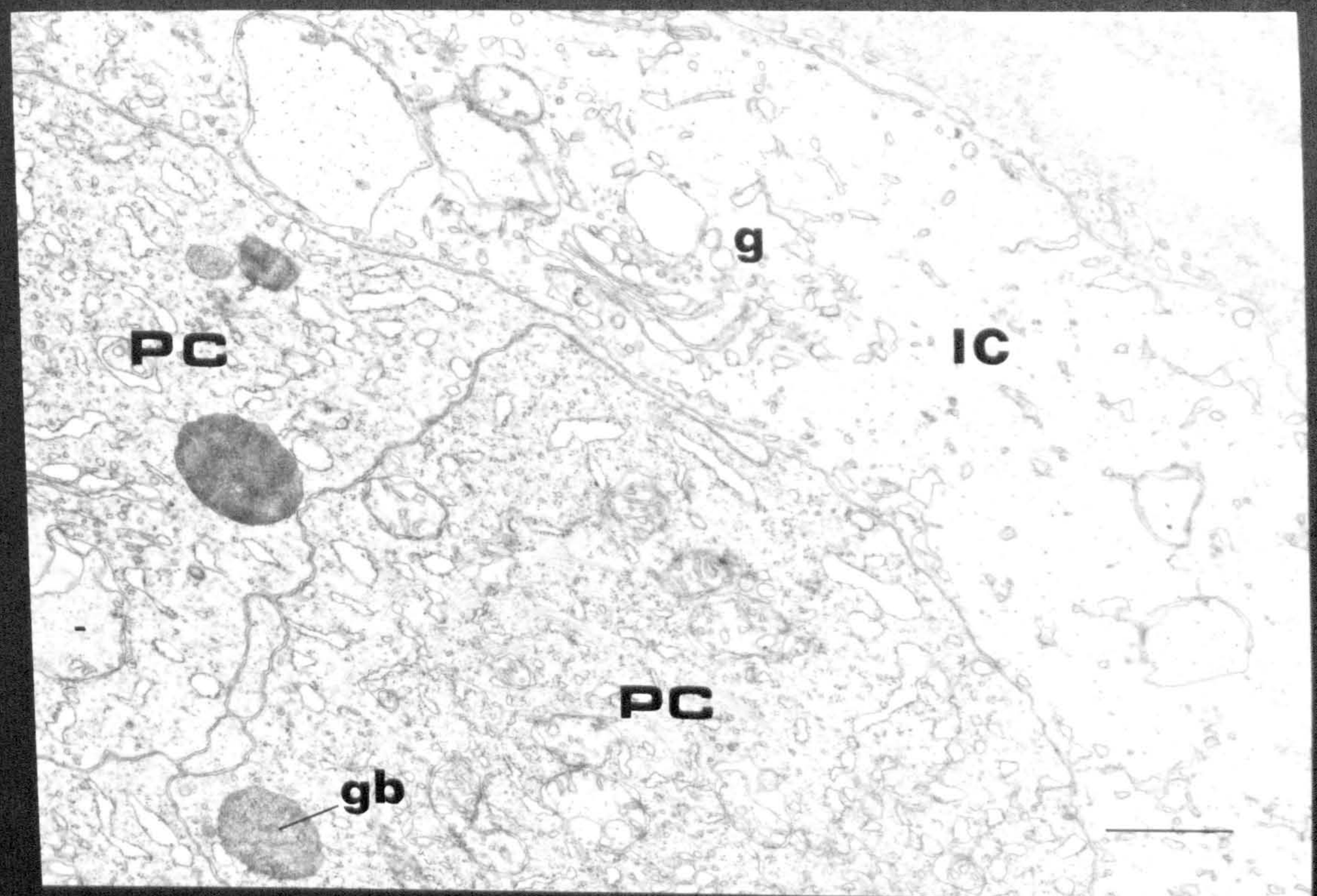
Plate 18

The photoreceptor cells give rise to lateral processes, which inter-digitate to form areas of possible synaptic contact. The gap junctions formed, may represent sites of electrotonic conduction.

mf - micro-filaments
p - photoreceptor cells
arrows - gap junctions

The scale line corresponds to a length of 0.5μm.

17



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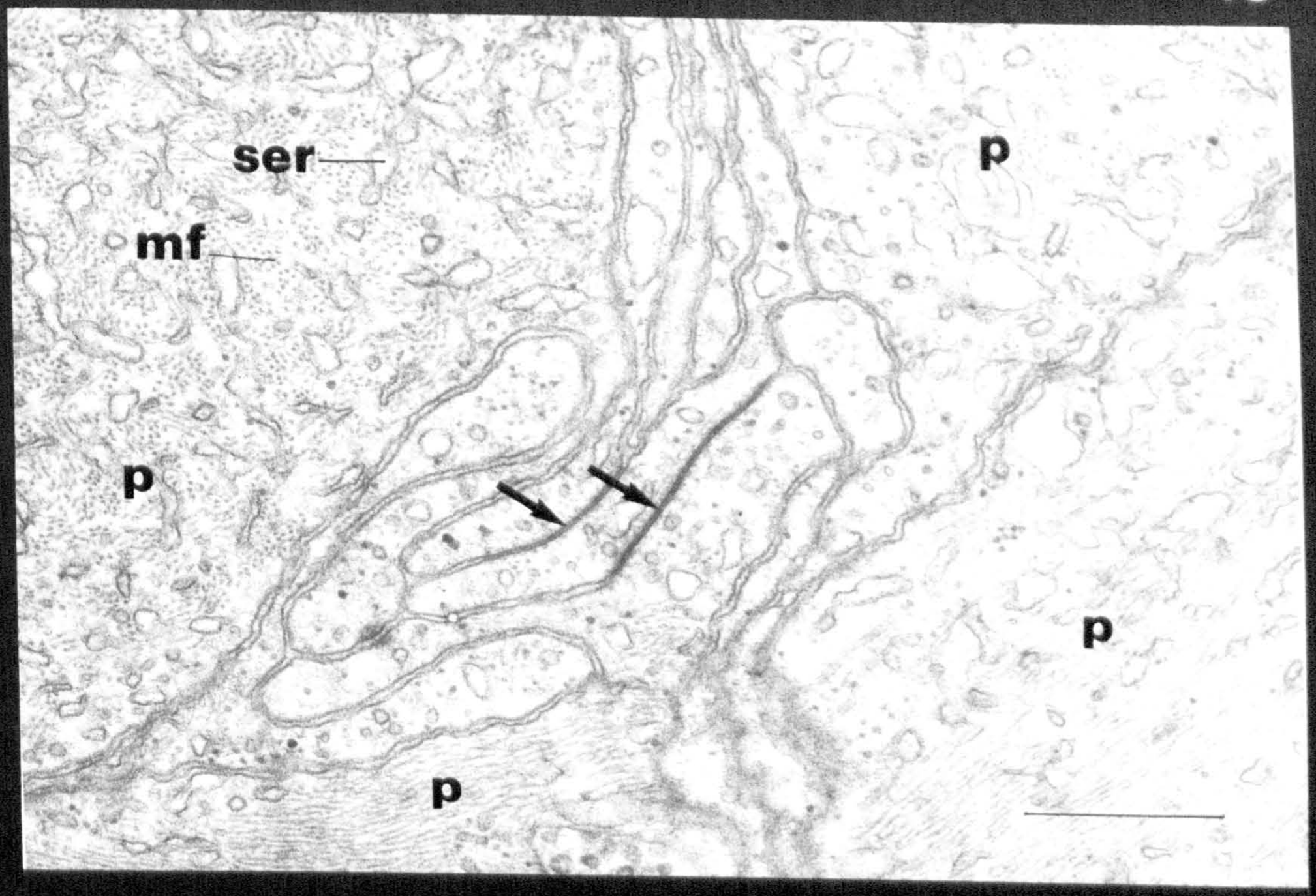


Plate 19

The basal processes of photoreceptor cells are characterised by smooth endoplasmic reticulum and extensive microfilament bundles. The electron density of the cytoplasm in adjacent photoreceptor cells can differ.
The cell at the top left of the micrograph gives rise to a lateral process, but its identity is unknown.

mf - microfilament bundles

ser - smooth endoplasmic reticulum

small arrows - trace the path of the lateral cell processes into the area of gap junctions

The scale line corresponds to a length of 1μm.

Plate 20

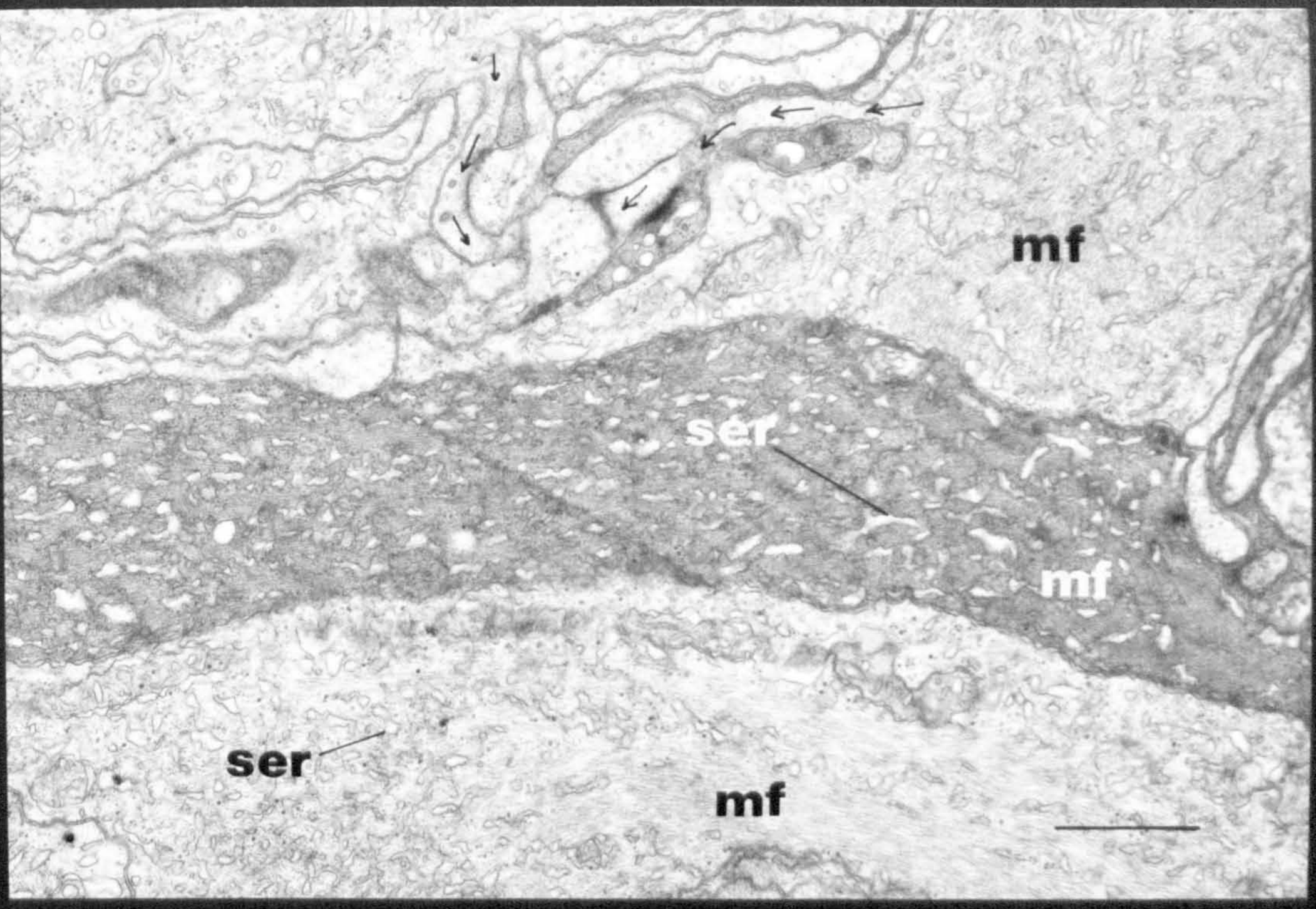
An enlargement of the junctional area in the above micrograph. The darker cell gives rise to processes, containing smooth endoplasmic reticulum, polysomes, and ribosomes, which form gap junctions with other photoreceptor cells and possibly another cell type.

PC - photoreceptor cell

arrows - gap junctions

The scale line corresponds to a length of 0.25 μm.

19



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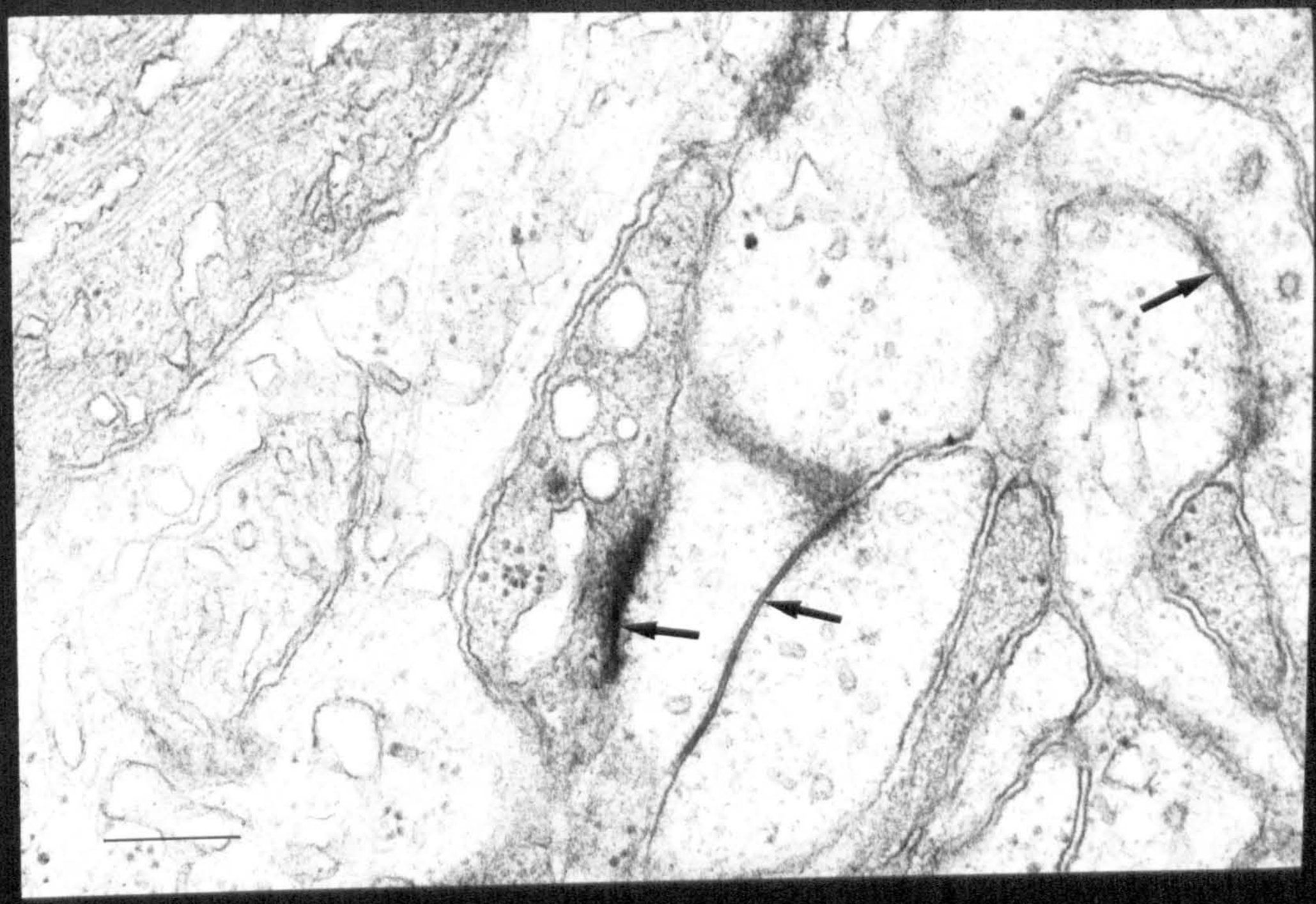


Plate 21

A disintegrating photoreceptor outer segment, which is characterised by a tubular arrangement of the distended lamellae. The micro-villi of interstitial cells occur throughout the area.

L - lumen

long arrow - 9+0 cilium

short arrow - micro-villi

The scale line corresponds to a length of 1 μm.

Plate 22

The pineal interstitial cells give rise to processes which extend into the peri-vascular space. The basal lamina of the pineal epithelium is well developed in comparison to that of the endothelial cells.

ca - capillary

en - endothelial cell

er - erythrocyte

p - pineal tissue

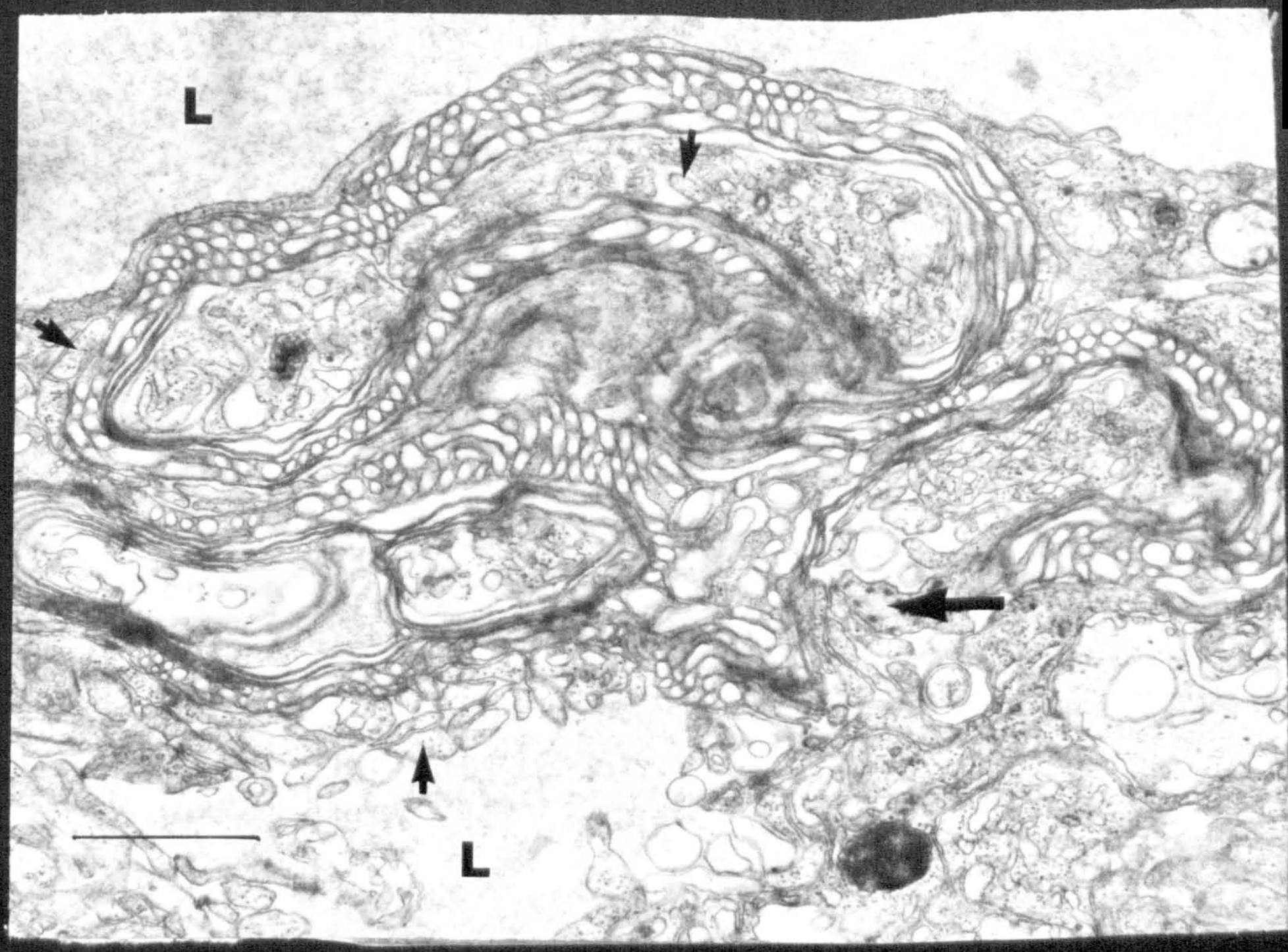
pvs - peri-vascular space

white arrows - basal lamina

small arrows - interstitial cell processes

The scale line corresponds to a length of 1 μm.

21



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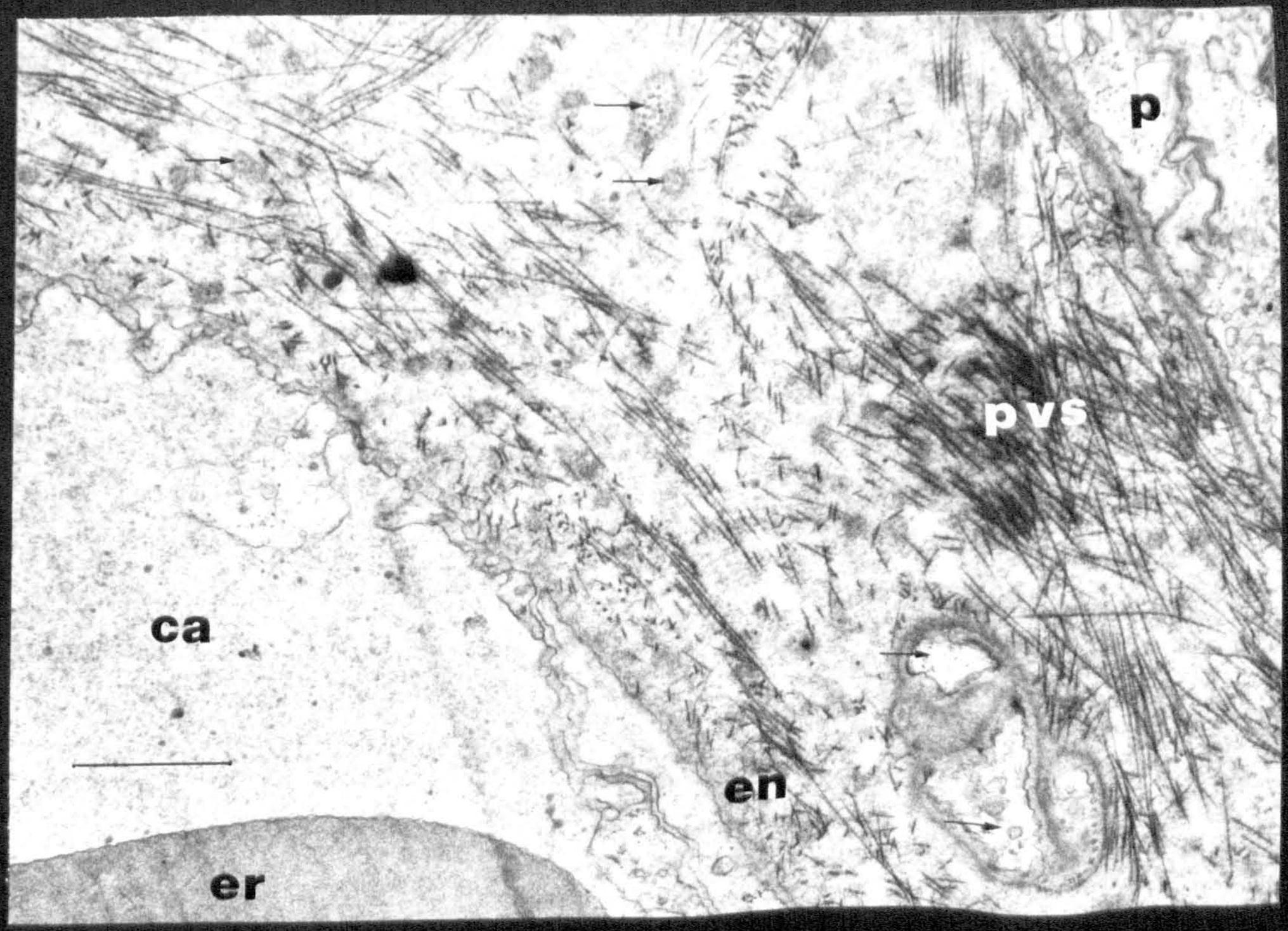


Plate 23

A cross section through the interstitial cell processes in the peri-vascular space.

bl - basal lamina

pvs - peri-vascular space

arrows - small vesicles

The scale line corresponds to a length of 1 μm.

Plate 24

A sagittal section through an interstitial cell process showing the extent of the basal lamina extension.

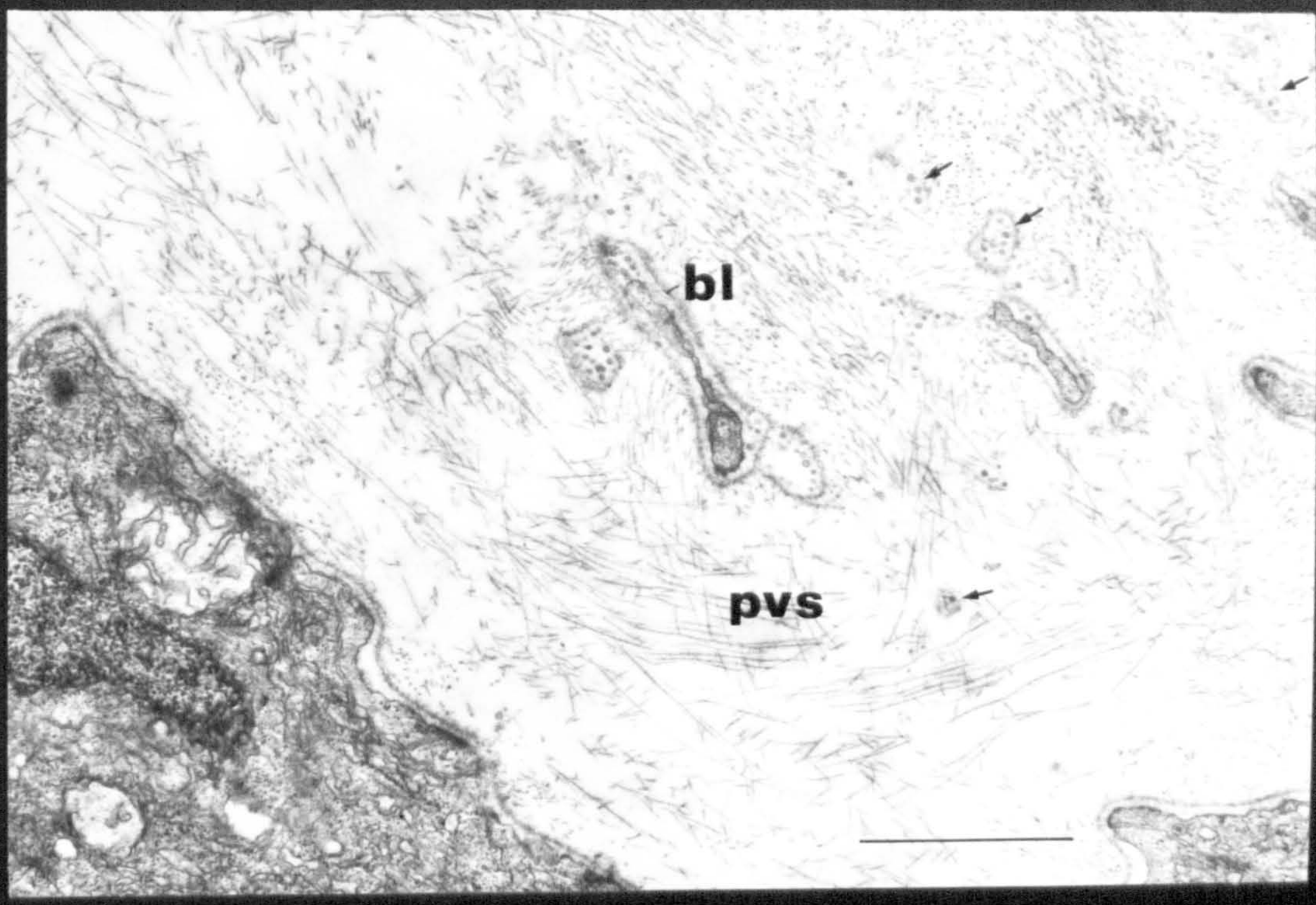
bl - basal lamina

pvs - peri-vascular space

arrows - small vesicles

The scale line corresponds to a length of 1 μm.

23



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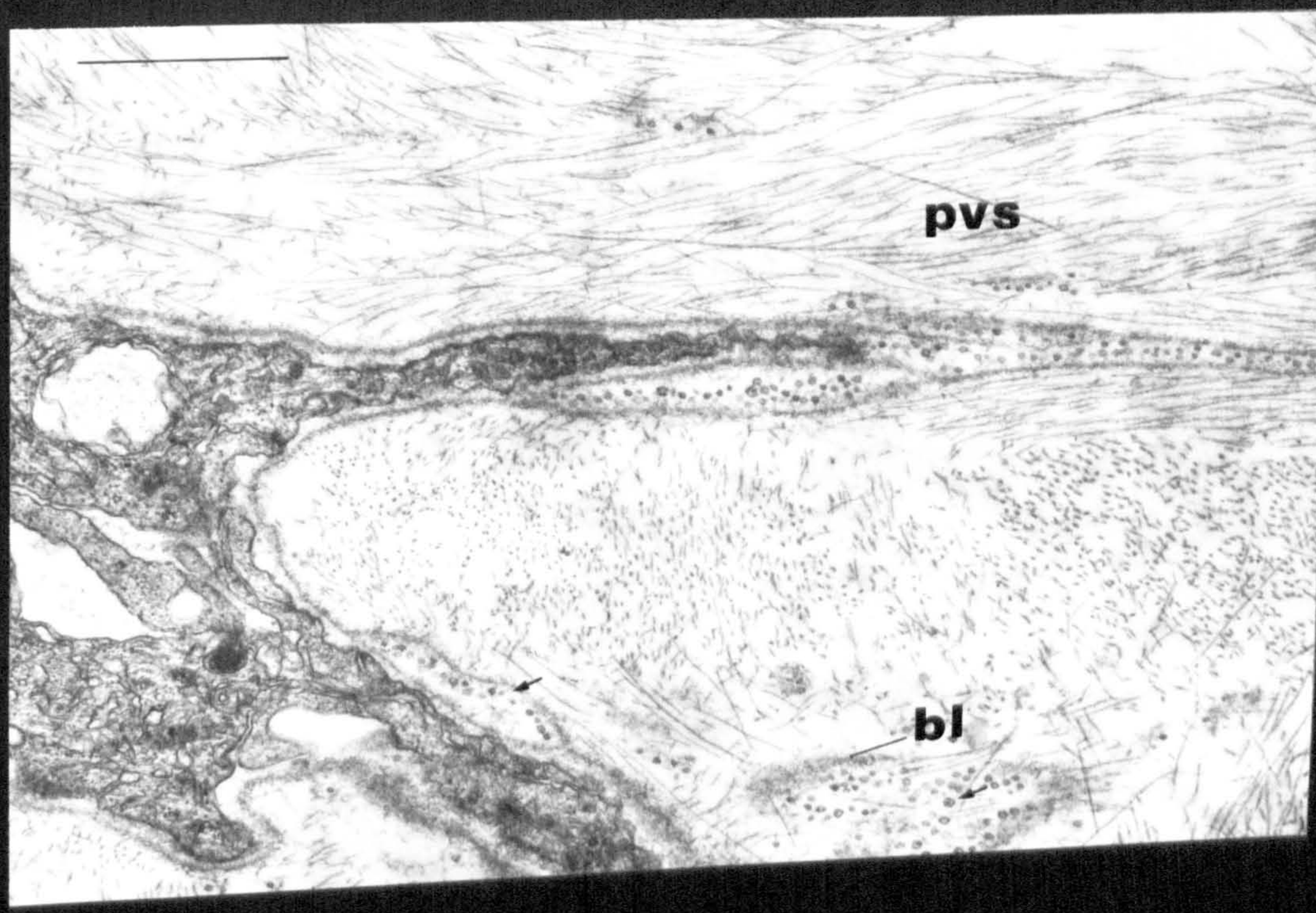


Plate 25

A large mitochondrion in the apical cytoplasm of an interstitial cell, which is situated close to an area of microvilli.

L - lumen

M - mitochondrion, interstitial cell

MV - microvilli

The scale line corresponds to a length of 0.5 μm.

Plate 26

The main body of interstitial cells often contains the dilated cisternae of smooth endoplasmic reticulum.

in - interstitial cell nucleus

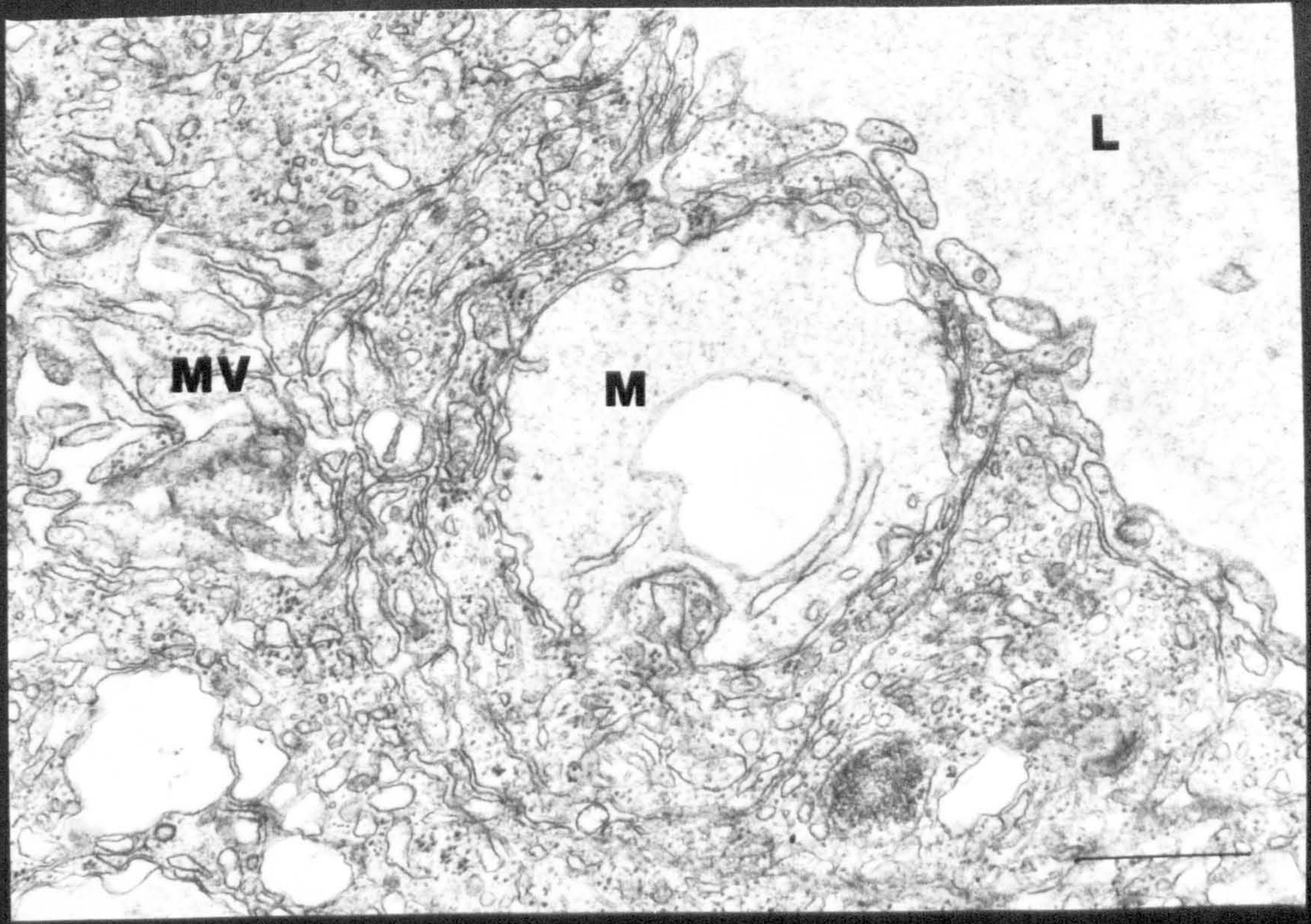
m - mitochondrion

ser - smooth endoplasmic reticulum, dilated

arrow - ribosomes

The scale line corresponds to a length of 0.5 μm.

25



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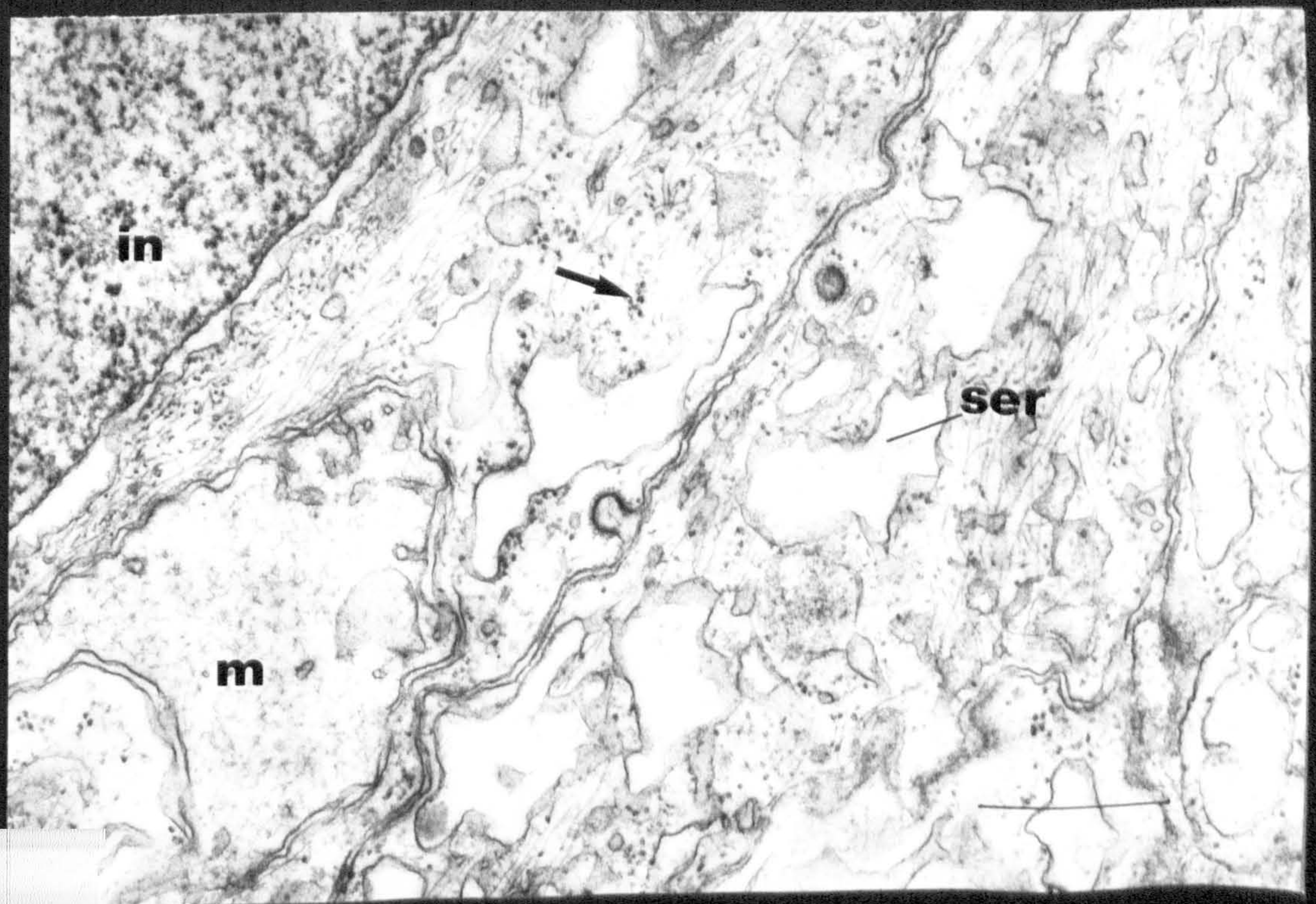


Plate 27

Interstitial cell nuclei close to the basal lamina, were occasionally found to be very electron dense and irregularly shaped. The position of these cells within the pineal epithelium, can be found in figure 42 .

Ni - nucleus of an interstitial cell

m - mitochondria

arrows - interstitial cell processes

The scale line corresponds to a length of 2 µm.

Plate 28

Interstitial cells with irregularly shaped nuclei of normal electron density, contained extensive rough endoplasmic reticulum and free ribosomes. The shape of the mitochondria in this cell differs from those in the cell above with the dark nucleus.

m - mitochondria

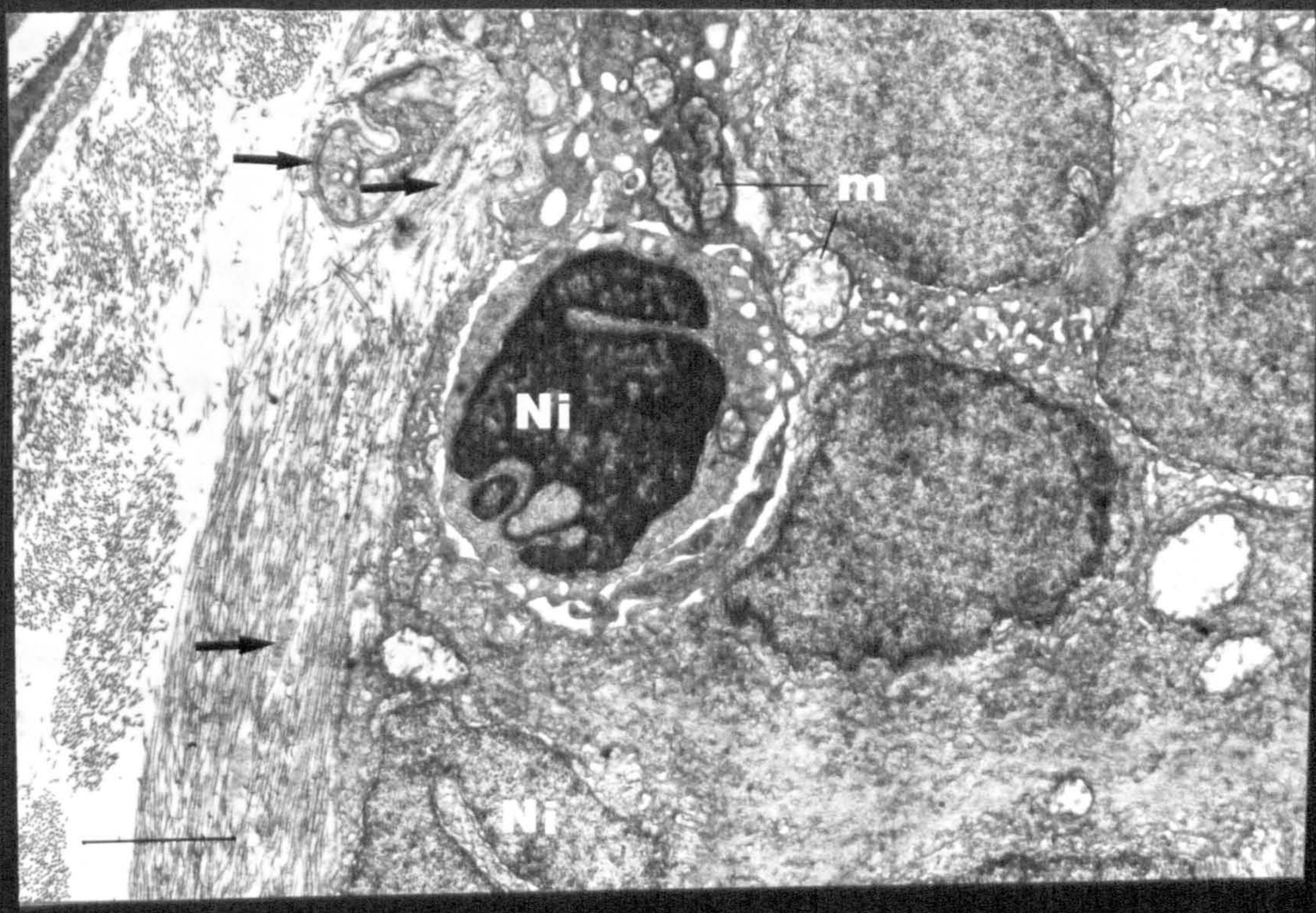
Ni - nucleus of an interstitial cell

r - ribosomes

rer - rough endoplasmic reticulum

The scale line corresponds to a length of 1 µm.

27



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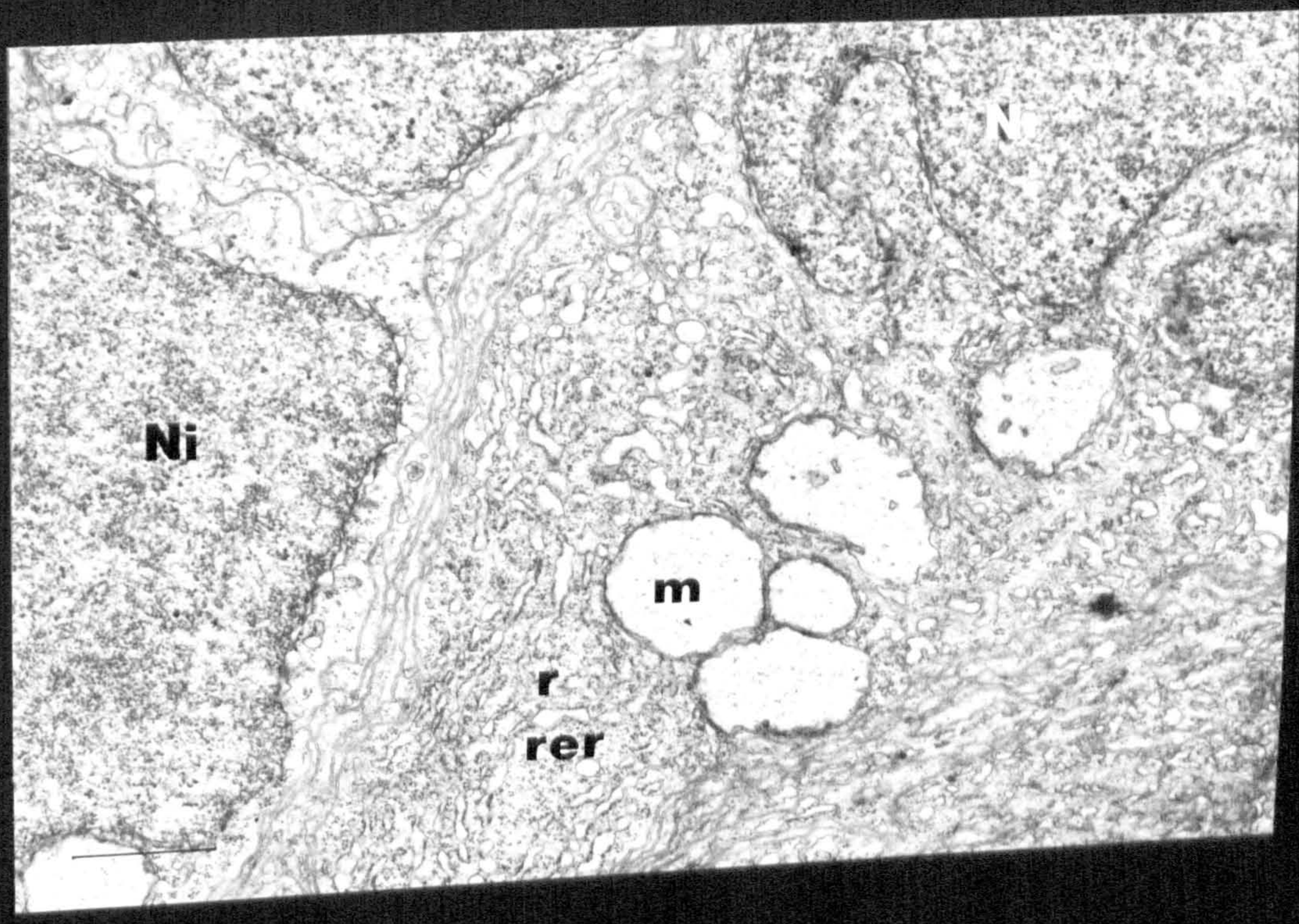


Plate 29

The epithelium close to the basal lamina is characterised by cell processes which are tortuosly arranged. It is not possible to be certain about the identity of the processes. Many of the processes contain clear or slightly electron dense vesicles. The vesicles are often found connected to the cell membrane, suggesting pinocytosis or exocytosis.

bl - basal lamina

white arrows - hemi-desmosomes

black arrow - vesicle attached to the cell membrane

The scale line corresponds to a length of 0.5 µm.

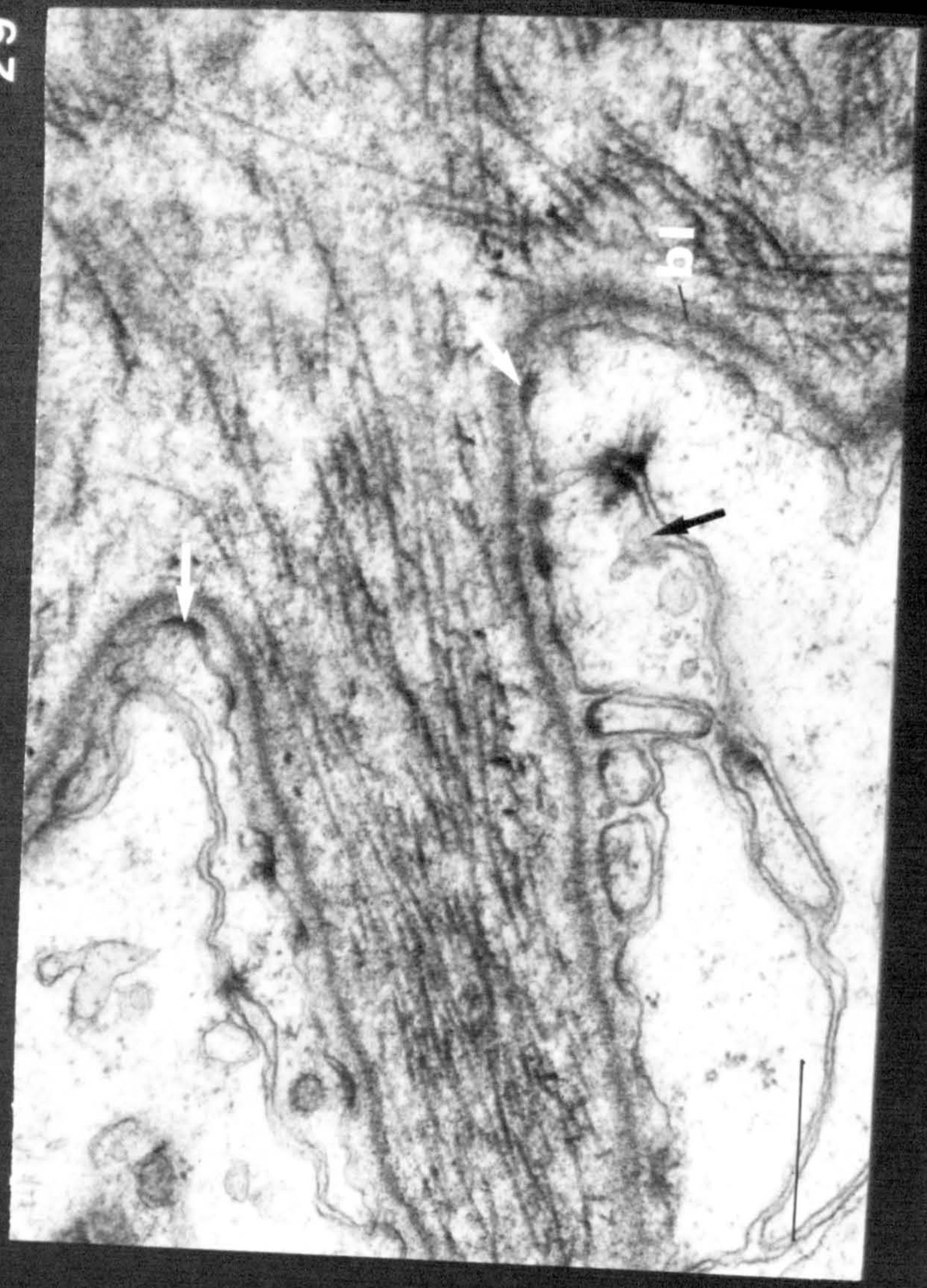
Plate 30

The vesicles are often concentrated in groups within the basal processes.

black arrow - clear vesicles

The scale line corresponds to a length of 0.5 µm.

29



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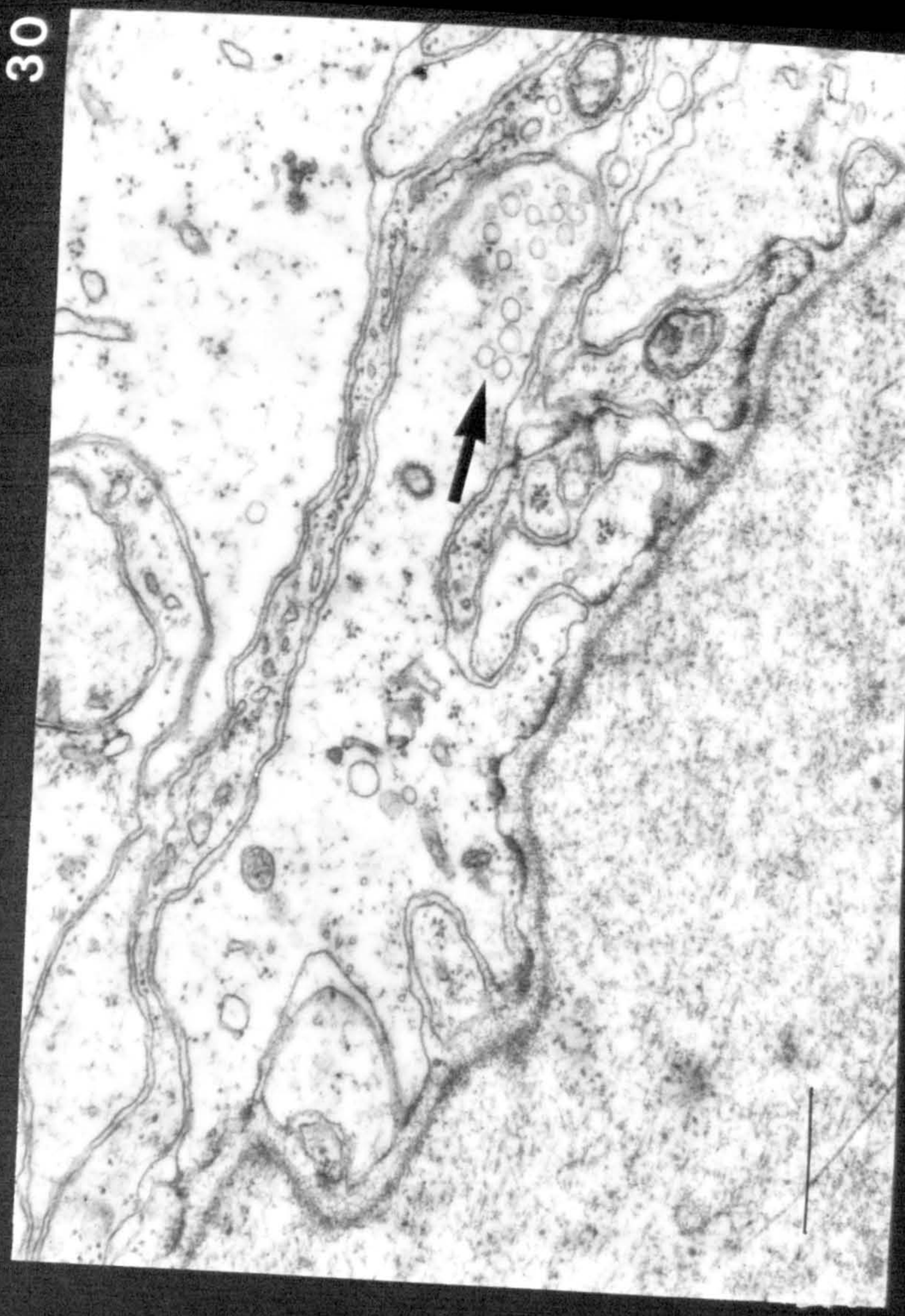


Plate 31

Electron dense areas occur within the basal processes. This micrograph shows electron dense tubular structures which have been demonstrated in other teleost pineals.

cv - clear vesicles

pvs - peri-vascular space

arrow - electron dense tubular structure

The scale line corresponds to a length of 1 µm.

Plate 32

Electron dense areas within a basal process, and close to cell extensions in the peri-vascular space.

pvs - peri-vascular space

long arrows - electron dense areas

short arrows - pineal processes

The scale line corresponds to a length of 1 µm.

31

32

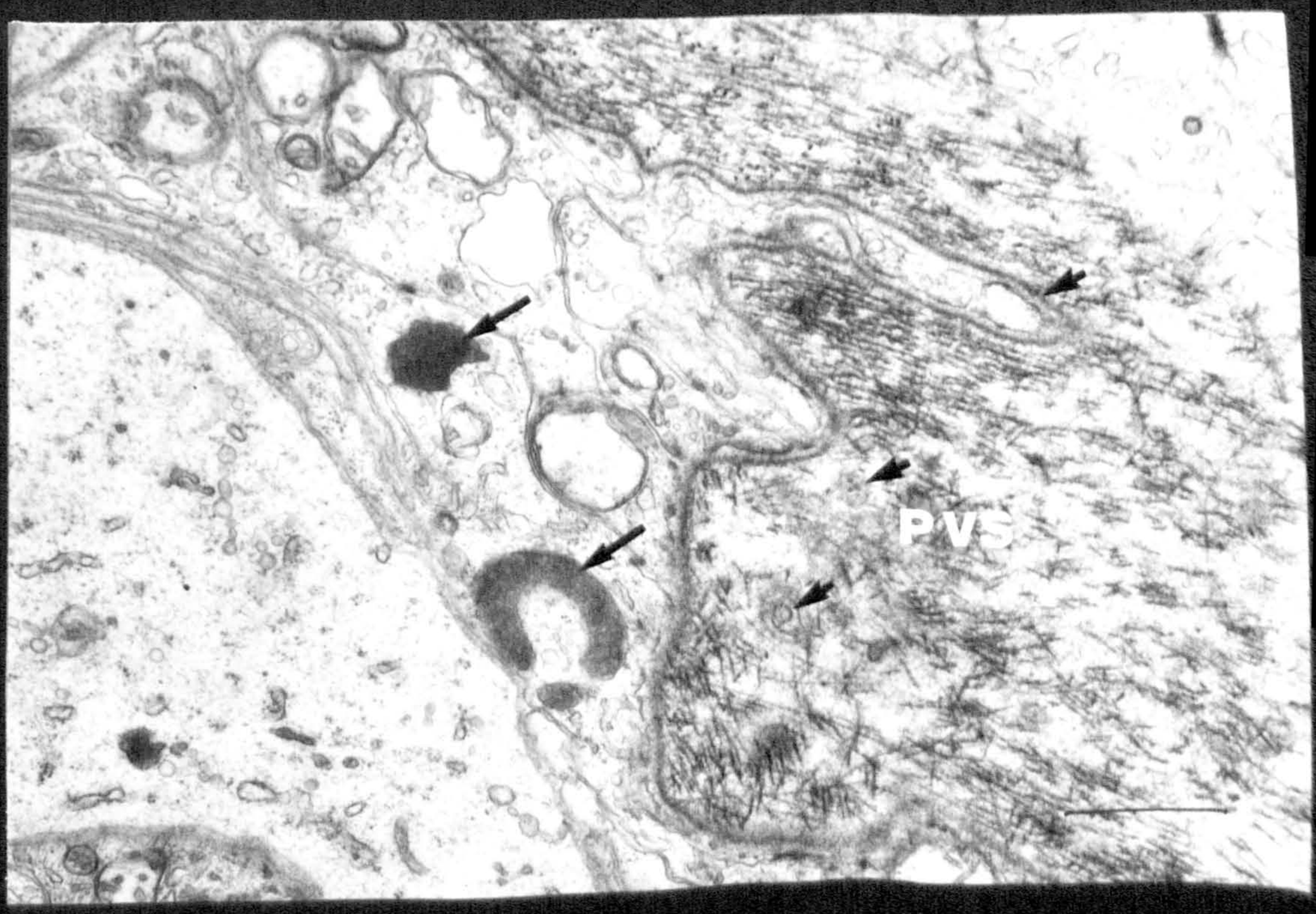


Plate 33

A section through the pineal epithelium, 1 μm, Methylene blue. This section shows two neurones which are located close to the lumen. Neurones take up stain to a lesser extent than either photoreceptor or interstitial cells.

Nn - nucleus of neurones

os - outer segment of photoreceptor cell

white arrow - neuropile

The scale line corresponds to a length of 10 μm.

Plate 34

An electron micrograph of the cell on the right (above). The cytoplasm is relatively electron lucent in comparison to photoreceptor and interstitial cells.

dcv - dense cored vesicle

er - endoplasmic reticulum

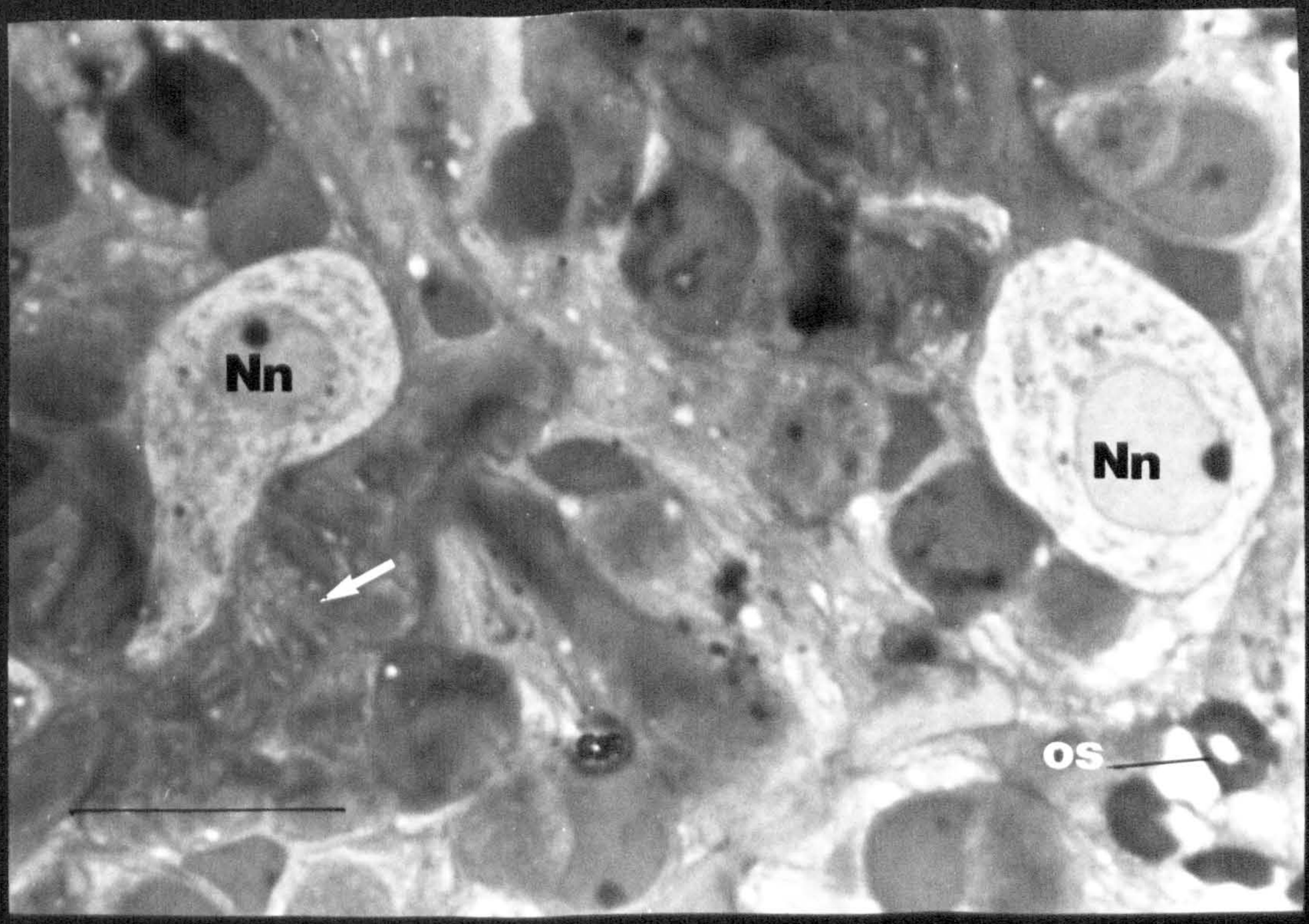
g - golgi body

m - mitochondria

Nn - nucleus of the neurone

The scale line corresponds to a length of 2 μm.

33



34

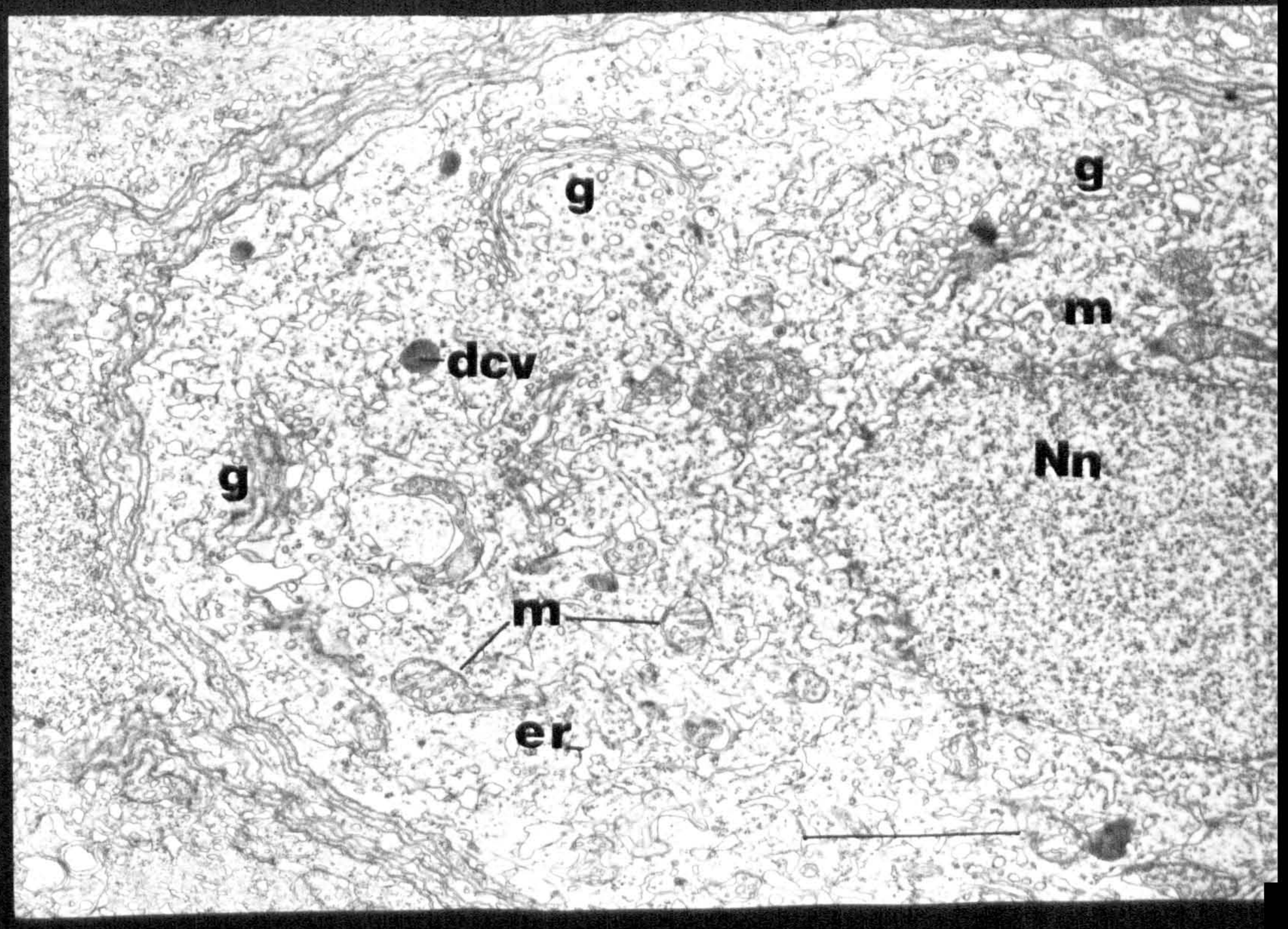


Plate 35

Sagittal section through 'simple' neuropile. The synaptic region between nerve cell processes and the photoreceptor processes is surrounded by interstitial cell processes. The narrow processes of the interstitial cells (small arrows) adjacent to the region of contact between the neuron and photoreceptor cell, highlight the difficulty in interpreting cross sections.

* - interstitial cell process
small arrows - interstitial cell processes
m - dark mitochondria
np - nerve process
pp - photoreceptor process
sr - synaptic ribbons
triangles - multi-vesicular bodies
thick arrow - microfilament bundle

The scale line corresponds to a length of 1 µm.

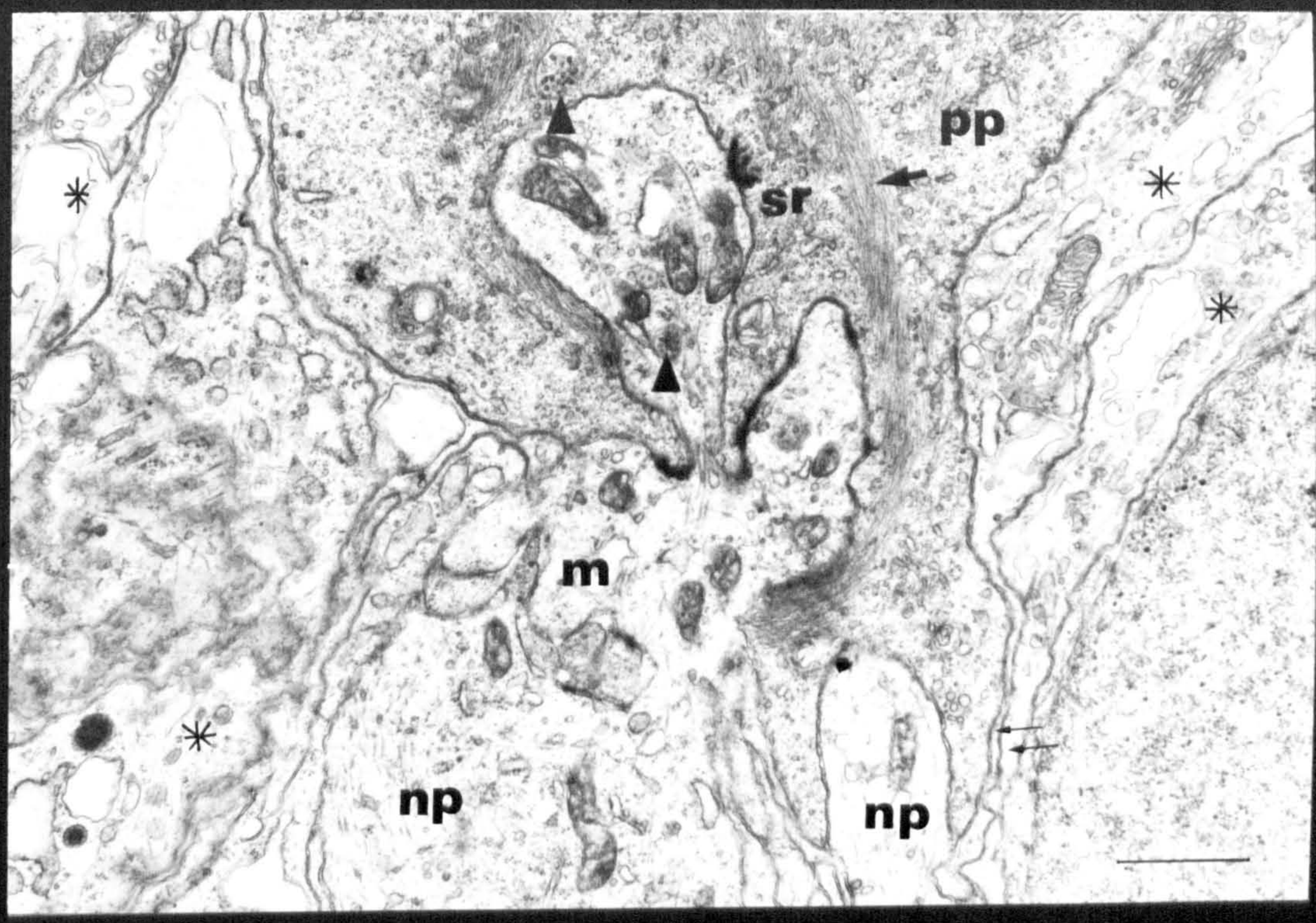
Plate 36

Cross-section through an area of neuropile. The processes of two photoreceptor cells are traced. Plate 35 indicates that the centre of the neuropile is a nerve cell process. The areas of close membrane contact, probably gap junctions, occur between photoreceptor cell processes.

triangle - multi-vesicular body
pp - photoreceptor cell process
small arrows - trace the path of one process
red arrows - trace the path of a second process
thick arrows - probable gap junction

The scale line corresponds to a length of 1 µm.

35



36

Plate 37

Sagittal section through neuropile showing the relationship between nerve and photoreceptor cell processes.

d - dendrite

white arrows - dendritic spines

m - mitochondria

mt - microtubules

e - electron dense material in the inter-cellular space

sr - synaptic ribbons (photoreceptor cell)

The scale line corresponds to a length of 1 µm.

Plate 38

Synaptic area between nerve cell and photoreceptor cell processes.

mt - microtubules

np - nerve cell process

pp - photoreceptor cell process

sr - synaptic ribbon

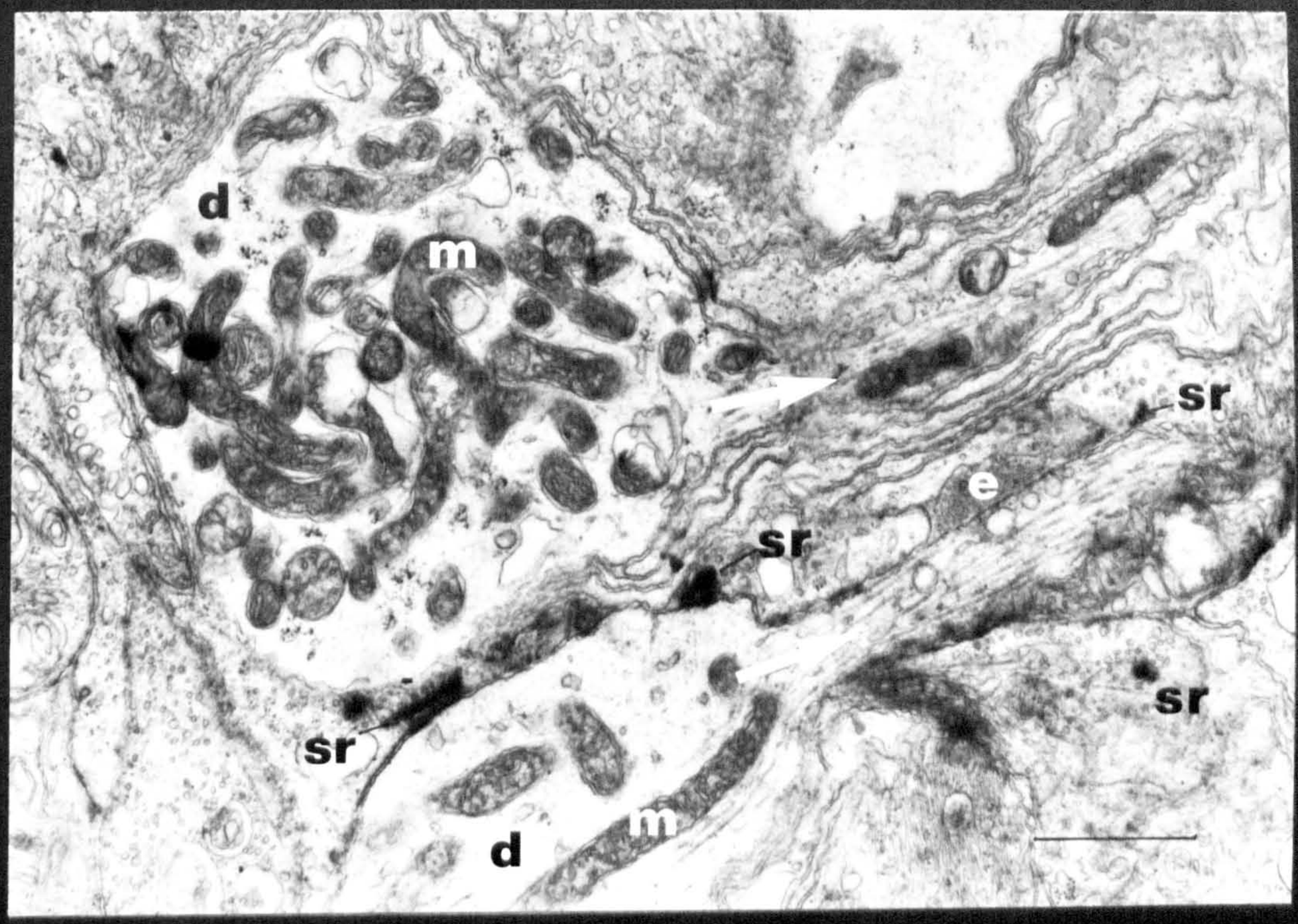
sv - synaptic vesicle

white arrow - pre-synaptic specialisation

open black arrow - post-synaptic specialisation

The scale line corresponds to a length of 0.25 µm.

37



38

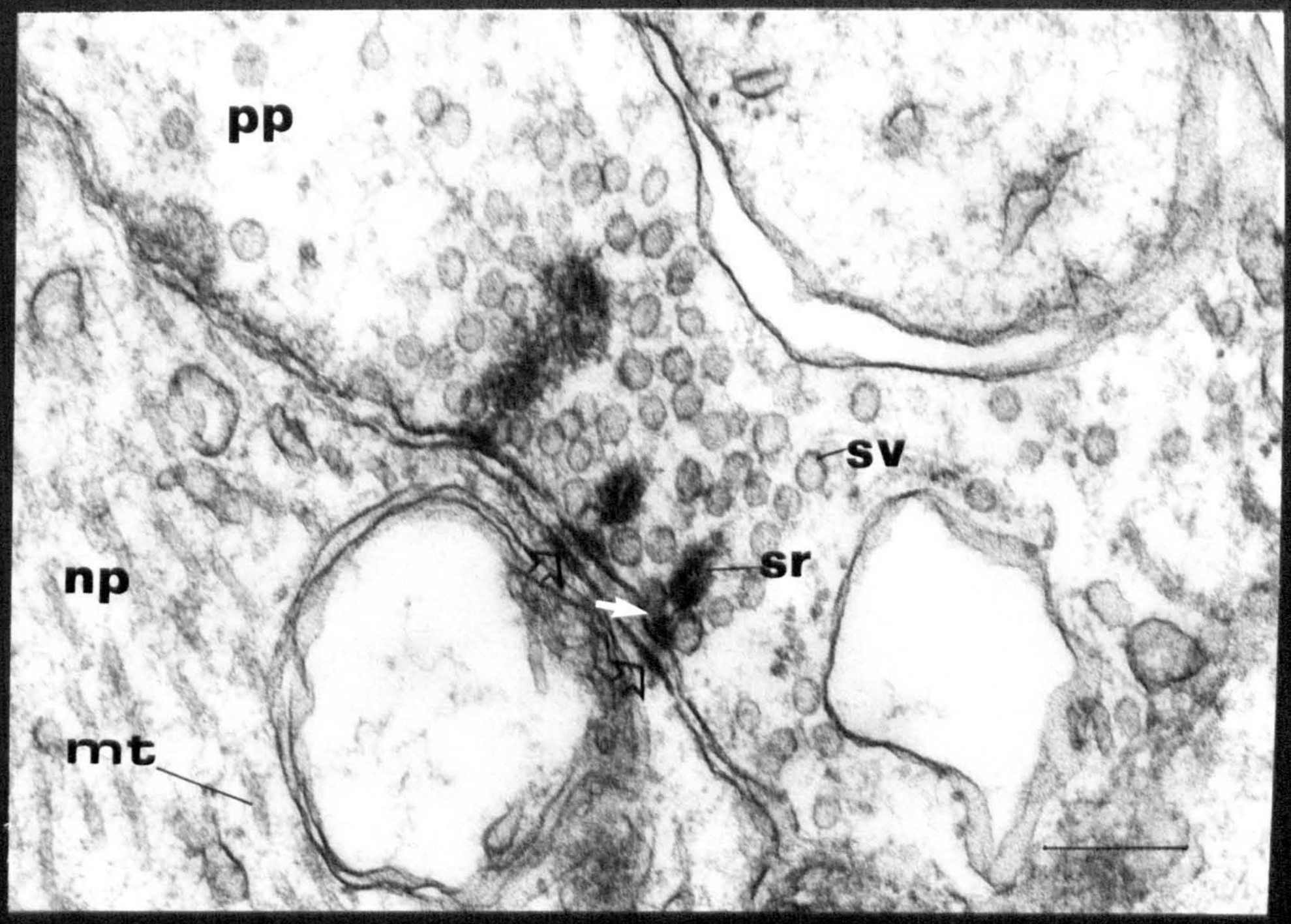


Plate 39

An area of neuropile with membrane specialisations between photoreceptor cells and nerve cell-photoreceptor cell.

m - dark mitochondria

np - nerve cell process

pp - photoreceptor cell process

triangle - multi-vesicular body

short arrow - synaptic ribbons, post synaptic specialisation

long arrows - membrane specialisations

The scale line corresponds to a length of 0.5 μm.

Plate 40

Basal process of a photoreceptor cell showing a large number of clear vesicles

pp - photoreceptor cell process

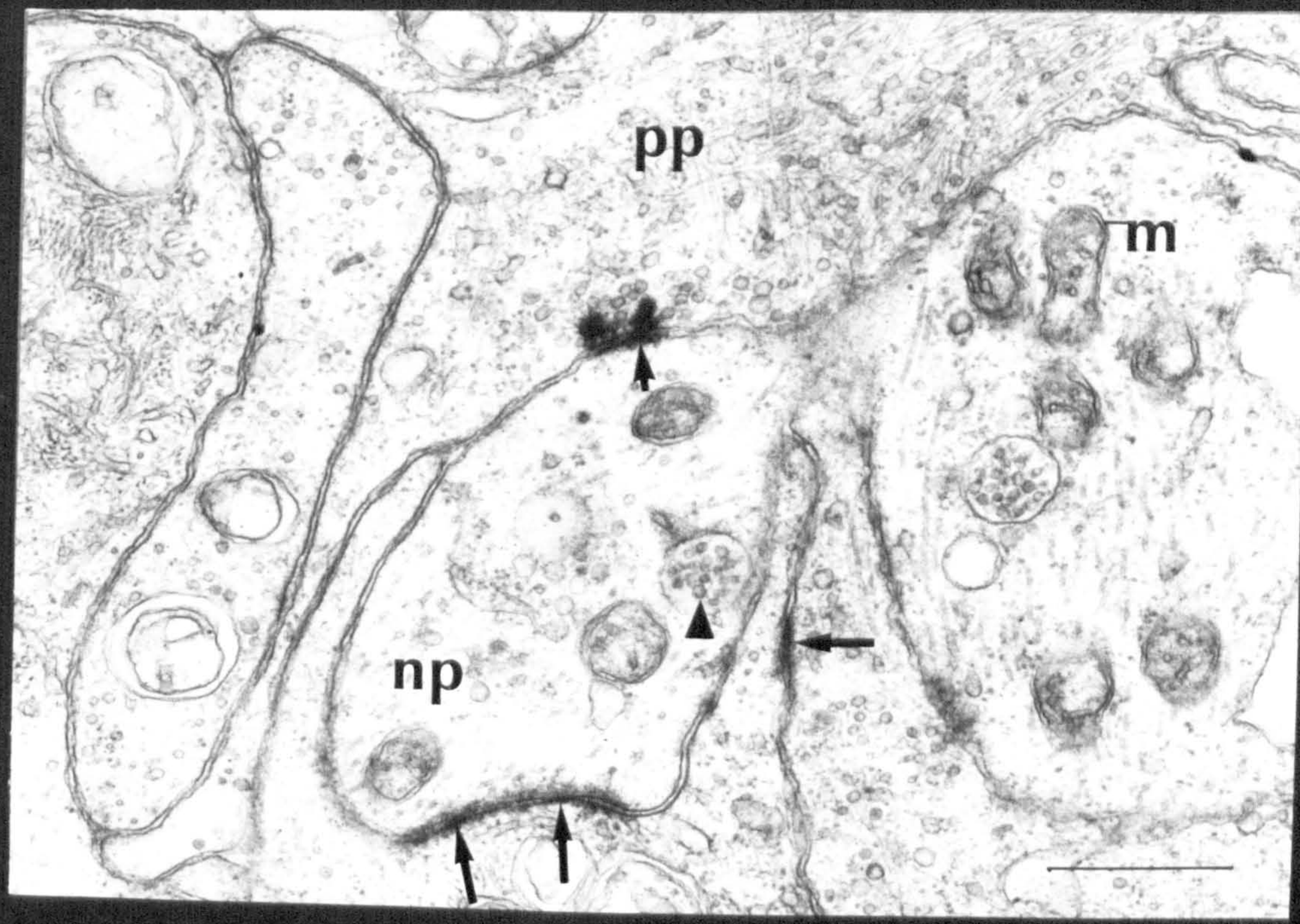
thin arrows - clear vesicles

long arrow - synaptic ribbon

short arrow - pre-synaptic specialisation

The scale line corresponds to a length of 0.5 μm.

39



40

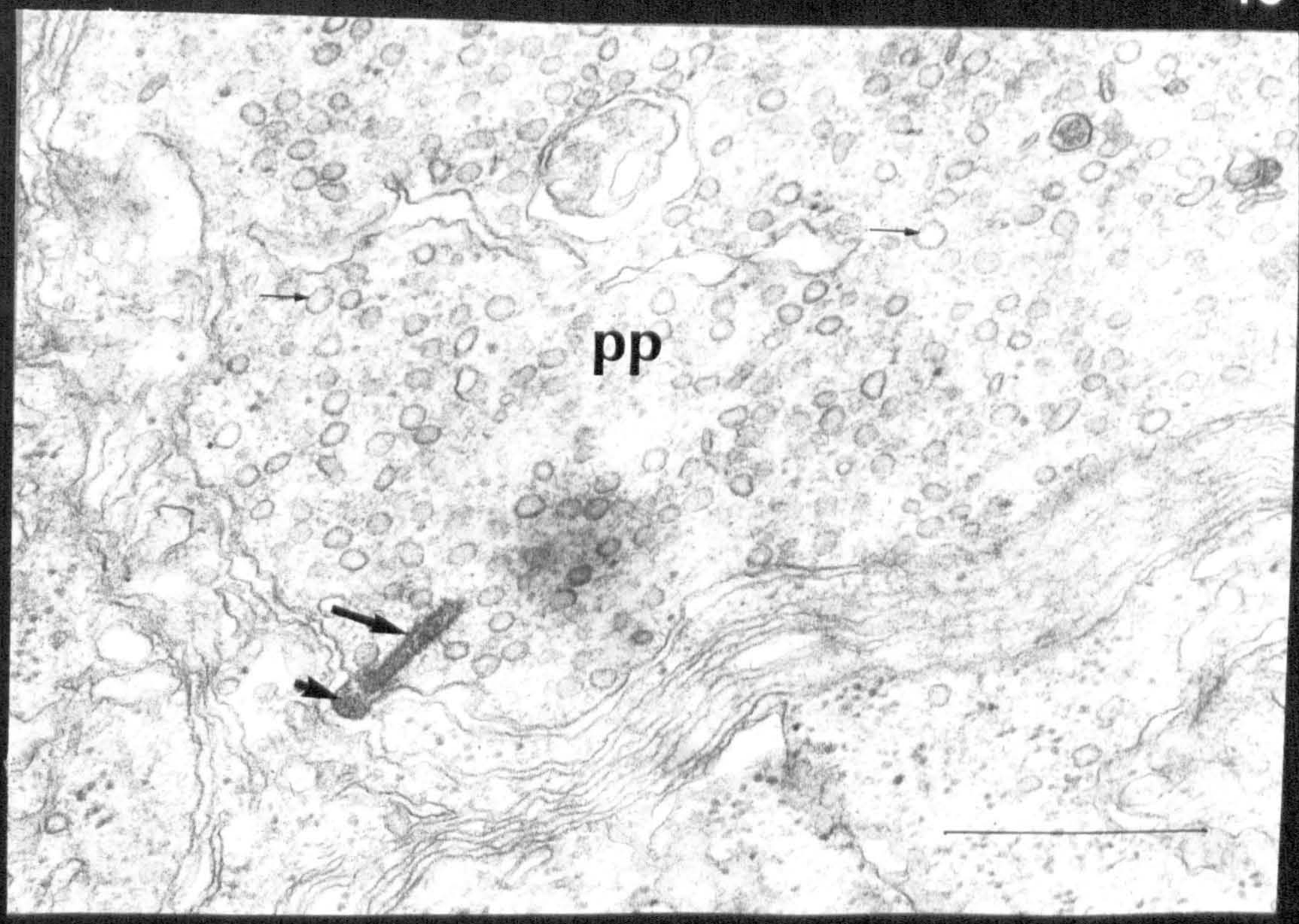


Plate 41

A scanning electron micrograph of the luminal surface of the pineal epithelium.

is - intraluminal septum

os - outer segment

The scale line corresponds to a length of 10 μm.

Plate 42

A scanning electron micrograph of an intraluminal septum.

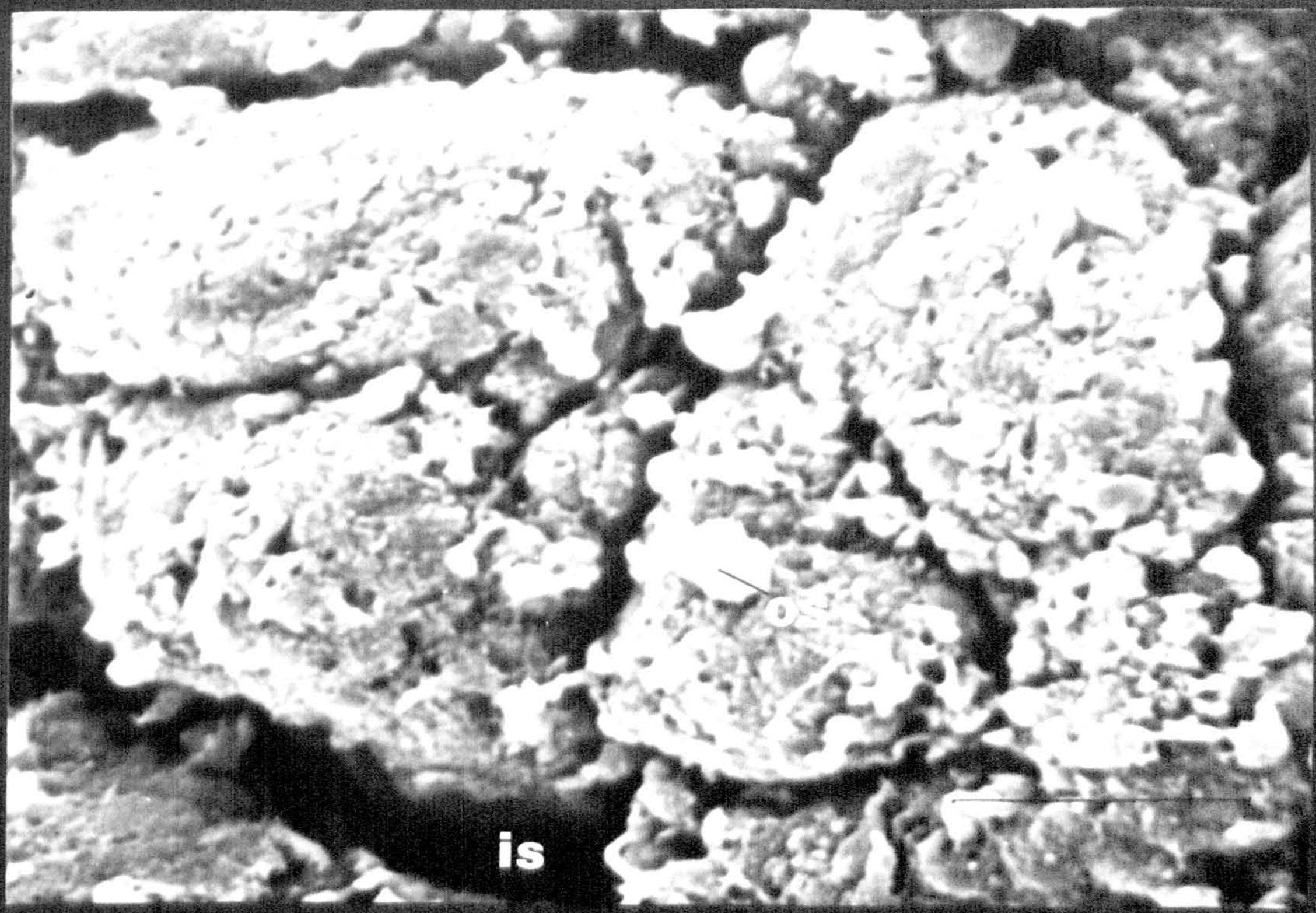
is - intraluminal septum

mv - microvilli

os - outer segment

The scale line corresponds to a length of 2 μm.

41



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